TEXAS REPORTS on BIOLOGY and MEDICINE

VOLUME 6, NUMBER 2 SUMMER, 1948

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FACTORS GOVERNING PROGNOSIS IN TUMORS OF THE BOWEL*

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It might be said that anything and everything that is done for the patient with a cancer of the large bowel affects the prognosis. Certainly there are many factors which influence the probability of a cure of a malignant lesion of the large intestine. The most important of these are: (1) the time of diagnosis; (2) the duration of symptoms; (3) the extent of growth of the neoplasm; (4) the size of the tumor; (5) the pathology of the neoplasm; (6) the location of the lesion; (7) the physical status of the patient; (8) the nature of the surgical procedures; (9) inadequate first treatment; (10) the attitude of the surgeon; and (11) the occasional occurrence of another primary malignancy. A particular endeavor has been made to evaluate the importance of some of these separate factors which govern the prognosis in cancer of the large intestine.

The time at which the diagnosis is made is a modifying factor of first importance. According to Heyd, the question of curability depends entirely on the diagnosis, and the diagnosis must be an essentially early one. The time the diagnosis is made has a direct relationship to most of the other factors influencing prognosis. This is a factor about which something can be done to increase the percentage of cures, for it is evident that a majority of carcinomas of the colon have been present from 12 to 18 months when the patient comes for surgical treatment. The symptoms of early cancer of the intestine are few and of a mild indefinite nature in incipiency, but they are usually of such degree that the alert physician will suspect the disease, and then by careful physical examination, proctoscopy and roentgenoscopy the diagnosis can be made.

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Swinton writes that in practically every case of proved carcinoma of the colon and rectum there can be elicited from the history the statement of a complaint which should have caused the suspicion of the probable presence of a lesion much earlier than it did, permitting earlier diagnosis. According to Stevenson, Moreton, and Seedorf, the roentgenologists can be depended upon to diagnose 95% of malignant lesions of the colon. In a review of the records of 190 patients with cancer of the colon, 126 or 66.3% of this series had palpable masses, and this is the most prominent physical finding. Seventy patients with lesions of the right colon had palpable growths, and in 16 no masses were detected. Fifty-six patients with lesions of the left colon had palpable tumors, and in 48 no masses were felt. It is seen that a larger percentage of the tumors of the right colon are palpable than are those of the left colon. The fact that two-thirds of these patients do not come to treatment until their tumors have grown so large that the neoplasm can be palpated is evidence that the cancer has been present for months, and that a diagnosis is being made late. Furthermore, most rectal cancers can be palpated by rectal examination, and all rectal and rectosigmoid lesions can be seen by the sigmoidoscope. Therefore, it seems logical to expect that more carcinomas of the large intestine should be diagnosed earlier.

The extent of growth of the lesion is a definite factor in governing prognosis. Early cancer of the colon and the rectum, as cancer elsewhere in the body, is a local lesion; and according to Wilkie, probably remains a local disease for many months to a year. In this stage of development it is readily amenable to surgical removal with the probability of permanent relief. Cancer begins in the mucous membrane, and it arises frequently from an adenoma or a papilloma. Its first method of spread is by extension and local infiltration. The next is by metastasis through the lymphatics and occasionally the cancer cells are disseminated by the blood stream. Fixation, perforation, obstruction, metastasis, operability and curability are all affected by the extent of the disease. Histologic studies emphasize the slow spread of cancer of the large

intestine. Harding and Hankins, in 118 postmortem examinations, found no apparent metastasis in 41% of the patients upon whom autopsies were performed. Such studies lend credence to the opinion that lymphatic invasion and distant venous metastasis seldom take place before the muscular coat of the intestine has been deeply invaded. Observations made by many interested in colon pathology support the opinion that a malignant lesion probably requires 12 to 18 months of growth to completely encircle the intestinal lumen. In the study of 100 resected specimens, it was noted that 45 of these had complete or practically complete encirclement of the intestinal lumen by the lesion, and the average duration of symptoms in this group was eleven and nine-tenths months. The long duration of symptoms before the patient comes to treatment emphasizes the need for study of the first symptoms of this disease. Probably most of the growths had existed a few months before suggestive signs or symptoms were in evidence. Most lesions, are, therefore, of a definitely longer duration than the patient's history would seem to indicate. Since the extent of the lesion is an important factor affecting the cure, it is an encouraging fact to know that the majority of patients with cancer of the intestine have lesions that are local, removable and curable for many months.

Consideration should be given to the size of the neoplasm, since it is somewhat of an index to the duration of the disease. The size is modified by four factors: the duration of the disease; the segment of intestine involved; the histologic type of carcinoma; the extensions with secondary inflammatory changes. All of these factors should be evaluated. It is quite evident that the longer the lesion has had to grow, the larger the tumor will be. The medullary cancers, which tend to produce the larger lesions, occur more often in the right colon; while the scirrhous carcinomas are usually smaller growths and are seen to predominate in the left colon. The average size of lesions of the right colon is 6.3 centimeters. The average size of lesions of the left colon is 5.5 centimeters. We would particularly call attention to the fact that the size of the growth is no criterion for the presence or absence of metastasis. The histological type definitely influences the size of the tumor as

the papillary and medullary cancers tend to produce the larger neoplasms. Symptoms do not always bear a direct relationship to the size of the lesion, for it has been observed that an acute obstruction was produced in the sigmoid by a lesion only 2 centimeters in size and by another 2½ centimeters in diameter. In contrast, some of the cancers of the right colon were 6 or 7 centimeters in size before symptoms became of a prominent nature. The fact that the lesion is found to be large does not necessarily mean that it is incurable. Some of the larger lesions were still local, resectable, and curable; while some of the smaller lesions had local and distant metastasis, and were incurable. Therefore, it should be emphasized that the size of the growth bears no constant relationship to the duration or character of symptoms, to glandular metastasis, or to curability of the neoplasm.

The physical status of the patient is another factor governing the prognosis of carcinoma of the intestine. All surgery is precluded for some because of diseases of either the heart, kidneys, or lungs or some other debilitating illness and only a restricted operation should be advised for some others because of the hazard of a more radical surgical procedure. The age of the patient is to be considered. Statistical data indicate that approximately 70% of patients with malignancies of the colon and rectum are over 50 years of age. Therefore, it is evident that cancer of the colon is in a large measure a disease of advancing age. It is the opinion of Rankin and Graham that due to atrophic changes in the tissues and lymphatics carcinoma of the colon is held in check and remains local for a much longer period in older patients. Larson says that metastasis occurs more often, rapidity of growth is greater, and that curability is less likely in the young. Shedden writes that the younger the patient the greater the tendency to a high grade of malignancy. Therefore, it seems logical to conclude that the age of the patient had a direct influence on the rapidity of growth, the extent of the neoplasm, the grade of malignancy, the time and incidence of glandular metastasis and the resectability and curability of the lesion. These facts justify the statement that a particular effort should be made to prepare the aged patient for resection so that they will not

be denied what surgery has to offer to them. Let it be said again that by adequate rehabilitating measures and careful selection of the proper surgical procedure, it will be found necessary to deny surgery to but few patients with resectable lesions. By the administration of parenteral fluids, dietary supplements, chemotherapy and infusions of whole blood or plasma, much can be done to prepare these patients for surgery, so that a removal of their lesions will not be unduly hazardous. Within the past year we have resected malignant lesions of the large intestine for 12 patients over 70 years of age, and one patient was 87 years of age.

The fact that approximately 10% of the carcinomas of the colon produce obstruction should be given consideration in a study of the factors governing the prognosis. This is particularly true since it is conservatively estimated that 20% or more of the patients with acute obstruction of the colon lose their lives. In a study of obstruction of the colon due to operable malignant lesions, Gregg and Dixon report the surgical treatment of 121 cases with a mortality of 34.7%. Time will permit only this observation, the mortality following primary resection of an obstructing carcinoma of the large intestine is prohibitive and, therefore, is never justified. Such a patient should have immediate surgical decompression of the obstructing large intestine followed by adequate rehabilitation. Then the resection can be performed with relative safety.

The location of the lesion deserves consideration. The symptoms, the physical findings, the duration of the disease, the size of the tumor, the pathology, the surgical procedure and the prognosis are all altered by the location of the neoplasm. The smallest caliber of the large intestine is in the rectosigmoid. The cancer often grows along the blood and lymph vessels around the inside of the intestinal wall, producing annular, constricting tumors. The small caliber of the bowel and the nature and development of the neoplasm here accounts for the fact that the first symptoms of the rectosigmoidal cancer are frequently those indicative of a partial obstruction. Statistical studies show that approximately 50% of all cancers of the large intestine are in the rectum, the next most frequent site is in the sigmoid, the third is in the rectosigmoid and the

fourth is in the cecum. Approximately 80% of all cancers of the large intestine occur in these four locations. The position of the carcinoma also has a direct bearing on its accessibility, the type of operation to be performed and the hazard of its removal. According to Vynalek, Saylor, and Schrek, the postoperative prognosis is best in right sided lesions.

There are four factors pertaining to pathology which influence the prognosis. They are the histologic type, the degree of activity of the malignant cells, the depth of muscle invasion, and the extent of lymph nodal metastasis. The papillary type is the most curable. It tends to grow on the mucous membrane surface and is slow both to invade the muscle wall and to metastasize to glands. The medullary adenocarcinomas have a tendency to produce large, fungating, ulcerating lesions, and they occur more often in the right colon. Due to their tendency to ulcerate into the intestinal wall, perforation, abscess formation and peritonitis are more frequently seen in this type, and metastasis occurs relatively often. The scirrhous carcinomas are the smaller, encircling, constricting lesions. They are seen usually in the left colon and obstruction is produced more often by this variety. The colloid carcinoma probably constitutes about 2% of the malignant lesions of the large intestine. This is a relatively slow growing type of cancer, but it has a poor prognosis due to the frequency of local extension and lymph nodal metastasis.

The degree of malignancy is the most important single factor relative to pathology governing the prognosis of carcinoma of the colon. The rate of growth, muscle penetration, lymph nodal metastasis, and curability are definitely affected by the grade of the neoplasm. Mayo and Lovelace, in a study of 885 cases of malignant lesions of the cecum and ascending colon, found that the lymph nodes were involved in only 22% of the cases in which the lesions were Grade I, whereas they were involved in 59% of the cases in which the lesions were Grade IV. The percentage of cures decreases as the grade of the malignancy increases. It is a hopeful fact observed in a study of statistical data that more than 60% of carcinomas of the large intestine have malignancies of low degree of cell activity.

The possibility of a patient remaining free of recurrence of disease is certainly modified by the depth of muscle penetration by the malignant cells. Careful study of a large number of specimens in regard to the depth of muscle penetration by malignant lesions shows that the percentage of good results decreases in direct ratio to the depth of invasion into the intestinal wall by carcinoma cells. In the study of a series of 190 cases, it was observed that approximately 80% had penetration of malignant cells to or beyond the serosa.

Lymph nodal metastasis is an important factor affecting prognosis. Detailed pathological study of resected specimens supports the opinion that lymphatic metastasis seldom occurs until there is deep penetration of the malignant cells into or through the intestinal wall. Therefore, metastasis to the lymph nodes is usually a relatively late occurrence. The above facts support the opinion that the postoperative progress is excellent in patients with low grade carcinomas of the colon which are unaccompanied by penetration of the musculature or lymph nodal involvement.

The nature of the surgical procedures are definite factors governing prognosis. After the diagnosis, the most important consideration is the percentage of operability or resectability of the neoplasm. After proper preparation and rehability of the patient, many surgeons of experience have now extended the field of resectability to between 70% and 75%. Lahey, in a recent article, reported a resectability of 83% for rectal neoplasms. By extending the field of resectability, certainly more patients have an opportunity of remaining well. The type and thoroughness of the operation performed is exceedingly important. Surgical extirpation is the only curative therapy for a patient with a carcinoma of the colon or rectum. I would emphasize that surgeons should be familiar with the different procedures so that they will be able to use the one best adapted for the individual case. Some patients are denied the probability of cure by inadequate and incomplete operations. Cauterization, fulguration or local excision of a carcinoma seldom results in a cure. To give to the patient with a cancer of the bowel the greatest probability of cure, a wide excision of the lesion with the regional gland bearing tissue is necessary.

While surgery of the colon to a degree can be regarded as standardized, certainly every case must be individualized and the operation modified to meet the conditions presented by each case.

Improper first treatment definitely influences the number of patients cured with carcinoma of the large intestine. A study of 167 cases of rectal cancer was made to determine the nature of treatment given prior to admission to the hospital. Fifteen patients had hemorrhoidectomies, nine were given injections for hemorrhoids, five had local cauterization of the lesion, ten patients had some other type of inadequate operation and 27 had some kind of medical treatment. It was seen by this review that 66 patients, or approximately 40%, had some type of inadequate therapy before their conditions were definitely diagnosed. Such improper first treatment delays a correct diagnosis and causes more patients to come to surgery late, at a time when many more of the lesions are incurable. In fact, incomplete operations unquestionably do harm by tending to disseminate the disease and by preventing the patient from coming earlier for adequate treatment.

The attitude of some physicians occasionally is discouraging. They assume that treatment is of but little avail and, therefore, that all cases of carcinoma of the large intestine are relatively hopeless. Only some ten days ago I saw a patient with a carcinoma of the rectum who had been seen some ten months before by a very good surgeon, and according to the patient, she was advised that there was nothing curative that could be done for her. Even when seen 10 months later, the patient still had a resectable lesion. We would express the opinion that a definite increased number of patients with carcinoma of the large intestine could be cured if the physician who first sees the patient would be alert to recognize the early symptoms of the disease and then advise immediate, thorough study of the case with institution of adequate surgical procedures.

In concluding this discussion pertaining to factors governing the prognosis of tumors of the large intestine, we would emphasize that it is well to remember that cancer of the colon is a local disease for months and metastasis occurs relatively late. A cancer of the colon is probably four times as curable

as a cancer of the stomach. A majority of the carcinomas of the intestine have been present 12 months or more when the patient comes for surgical treatment. The diagnosis is not particularly difficult. The symptoms of early disease are only suggestive, but when the condition is suspected the diagnosis can be made by physical examination, proctoscopy and roentgenoscopy. The size of the tumor is a poor index to curability. The size and fixation of lesions are due often to inflammatory changes. Regional lymph nodes are more often inflammatory than malignant. The lesion may not be incurable even though there is some extension of malignancy to regional structures. It is evident that cancer of the large intestine is in a large measure a disease of advancing age, for 70% of these patients are over 50 years of age. The poor risk patients can and should be rehabilitated and then given a chance of cure offered by surgery. There are four factors in regard to pathology which deserves consideration: the histologic type, the degree of activity of the malignant cells, the depth of muscular invasion, and lymph nodal metastasis. The pathology has a very important bearing upon the prognosis and the most important of these factors is the grade of the malignancy.

The occasional occurrence of another primary carcinoma of the intestine should be given consideration in reviewing the factors governing prognosis in tumors of the bowel. In the study of 306 carcinomas of the large intestine, it was found that 2.6% of these patients had 2 primary lesions of the bowel at the same time. Our observations would lead us to the conclusion that approximately 3% of the patients who have had one carcinoma of the large intestine will later develop another carcinoma of the large bowel. We have had one patient within a period of 12 years that had what was apparently 5 primary carcinomas of the large intestine. Another patient had 3 primary carcinomas within a period of 15 years.

The patient has no chance of cure except by extirpation of the lesion. The larger the number of resections performed, the more patients there will be who are given a chance of cure. Therefore, every reasonable effort should be made to remove or resect the carcinoma. Such an attitude assumed by the surgeon will definitely extend the field of operability and

will increase the ultimate percentage of cures of all cases seen. Surgery can be performed in stages when there are existing complicating factors and then multiple stage procedures will extend the field of operability and lower the mortality. A fact worthy of emphasis is that by an earlier diagnosis achieved in some cases the proper preparation of the patient, progress in anesthesia, advances in surgery and better postoperative care, the field of resectability has now been extended to probably about 70% or 75% of all patients seen. It is gratifying to know that 50–60% of patients surviving resections will be alive and apparently free of disease five years following surgery. In a series of 190 patients with cancer of the colon, removal of the tumor was accomplished for 66.6% of the patients operated upon. Seventy-one per cent of the patients who survived surgery lived more than 3 years, and 62% lived more than 5 years. This study emphasizes the fact that a carcinoma of the large intestine is not a hopeless disease and that radical surgical removal of the involved intestine has much to offer the patient with this pathologic entity.

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EXPERIMENTALLY PRODUCED LIVER TUMORS*

W. A. SELLE

Liver tumors can be produced in animals by several chemical agents. The first description of atypical growths produced in animals by chemical means was published in 1906 by Fischer, who employed a well-known dye, scarlet red. The tumors which resulted by the injection of this dye were histologically indistinguishable from cancer but, according to the early reports, they invariably receded. Many laboratories soon confirmed and extended Fischer's observations; the number of carcinogenic dyes was greatly increased. However, the discovery in 1918 by Yamagiwa and Ichikawa that coal tar when applied to the skin of rabbits or injected subcutaneously produces true malignancy, shifted the emphasis from the azo dyes to coal tar and coal tar preparations. The first conclusive demcnstration that azo dyes produce cancer of the liver of animals was published in 1935 by Sasaki and Yoshida who fed the dye, o-aminoazobenzene. A year later Kinosita (1936) published his findings on the carcinogenicity of another azo dye, p-dimethylaminoazobenzene. This dye is usually referred to as "butter yellow," since it was at one time used for coloring oleomargarine.

The original experiments on butter yellow involved diets composed mainly of rice flour, which, of course, is deficient in protein. It is perhaps significant that the discovery of this hepatic carcinogen was made in Japan where this cereal is the chief dietary constituent. Rats fed rice flour containing 1 mg. of butter yellow per gram of flour develop within 3 or 4 months degenerative changes of the liver, consisting chiefly of the hobnail type of cirrhosis; cancerous nodules appear in about 5 or 6 months. Death usually occurs by the seventh or eighth month.

Azo dyes are toxic; the animals usually lose weight, and ordinarily 10% to 15% succumb before tumors appear. The

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addition of protein (10% casein) increases survival, diminishes the frequency of cirrhosis and tends to prevent carcinogenesis.

The subcutaneous injection of p-dimethylaminoazobenzene in oil also produces liver cancer in 5 to 6 months. Intravenous injection of the dye in emulsified oil, however, does not, nor are tumors produced when the dye is applied directly to the skin. Many other compounds, mostly related to o-aminoazobenzene or to p-aminoazobenzene, have been tested for carcinogenicity; with but one exception, however, none of the derivatives has proven to be as potent as these original hepatic carcinogens.

Accompanying the changes in the liver, the spleen enlarges. Removal of this organ or blockage of the reticulo-endothelial system also greatly delays the onset of hepatic tumors. In addition to changes in the liver and spleen, there is a tendency toward gastric papillomatosis (Hoch-Ligeti, 1946). However, cancer of the stomach or intestines as a consequence of feeding the azo dyes has never been reported; this is in contrast to other potent carcinogens, such as methylcholanthrene.

The rat is by far the most susceptible animal to azo dyes; the mouse is next. This laboratory has tried unsuccessfully to produce liver tumors in guinea pigs, rabbits and chickens; we are now attempting to produce them in monkeys.[1]

Several chemical agents besides the azo dyes are effective hepatic carcinogens. Some of these are 2-acetylaminofluorene, an insecticide which produces tumors similar to those resulting from the azo dyes; (2) carbon tetrachloride, which causes extreme cirrhosis but no tumors in rats, produces hepatomas in mice; (3) selenium, a rare element, when ingested in amounts of 5 or 7 parts per million, produces severe cirrhosis and low grade carcinomas in rats after about eighteen months. None of these chemicals, however, produces tumors as consistently and in so short a time as the azo dyes.

As stated, the production of liver cancer by chemical means is generally preceded by varying degrees of liver damage and cirrhosis. The dietary agents which retard hepatoma formation also protect against liver injury in the absence of carcinogenic

[1]This work has been supported by a grant from the Sugar Research Foundation, Inc., New York.

compounds. In fact some workers believe that the primary effect of hepatic carcinogens is damaging rather than stimulating and that the riotous proliferation subsequently following is atypical regeneration.

Since varying degrees of liver cirrhosis can be produced in rats simply by feeding diets high in fat and low in protein, the possibility of hepatic tumors in the absence of carcinogenic agents arises. Many laboratories have attempted to produce liver cancer under such conditions. No one, however, has yet reported production of liver tumors in the absence of the carcinogen; hobnail cirrhosis may be extreme, nevertheless, cancer does not result.

While the azo dyes make it possible to study hepatic carcinogenesis quantitatively, there is still no evidence to suggest whether hyperplasia results directly from stimulation by the carcinogen, or in consequence of atypical regenerative process following degenerative changes. Kinosita (1937, 1940), the discoverer of p-dimethylaminoazobenzene, maintains that there are some degenerative changes. According to him, liver tumors are of two main types: Cholangiomas and hepatomas; the hepatomas are further divided into (1) solid, (2) aveolar, (3) trabecular, and (4) adenomatous types.

Orr (1940) is of the opinion that the primary changes brought about by p-dimethylaminoazobenzene on the liver are destructive and that the proliferation of liver and bile-duct cells is of the regenerative type. Supporting this claim he states that the earliest evidence of proliferation is usually found in the connective tissue of the portal tracts and not in the parenchymatous cells. Granulation tissue from the portal tract extends into the substance of the lobule; in addition, nearby liver cells show definite degenerative changes. Orr emphasizes that while the events taking place in the hepatic tissue are indistinguishable from those following administration of non-carcinogenic agents, which produce degeneration and necrosis in short-term experiments and cirrhosis after longer administration, cirrhosis itself does not lead to cancer. He recognizes three forms of liver tumors; bile-duct carcinoma (cholangiomas), bile-duct cystadenoma, and liver-cell carcinoma (hepatoma).

Opie (1944), on the other hand, states that hyperplasia of the liver following butter yellow administration occurs in the absence of cirrhosis or other widespread destruction. Hé reports diffuse and focal areas of hyperplasia in both parenchyma and newly formed duct tissue. Opie describes four classes of liver tumors: (1) trabecular hepatomas, (2) adenohepatomas, (3) cyst-adenomas, and (4) cholangiomas.

Nutrition in Experimental Cancer

Tumors in General

Few subjects concerning cancer have received so much controversy as has nutrition and tumor formation. In fact, our present knowledge of diet and cancer tends to pose more questions than it answers.

While it is difficult to visualize nutrition or diet, *per se*, as a major factor in tumor formation, unless, perchance, substances such as azo dyes are present in the diet, statistical studies of Hoffman (1937) and others suggest that individuals who develop cancer tend to eat more and are in general in better nutritive condition prior to cancer than are normal individuals, or individuals who are underfed and underweight. Likewise, observations in many laboratories have established that caloric restriction and certain dietary constituents alter the development of many spontaneous and induced animal tumors. In order to limit this discussion, the ensuing comments will be concerned chiefly with the caloric aspects of the subject.

Once formed, cancerous tissue grows and increases in weight although the host's body loses weight. In fact, transplanted tumors of the mouse are established as well in animals fed inadequately as in well nourished animals, but the growth of these transplanted tumors is retarded in malnourished hosts. Since tumors grow when the animal is in negative nitrogen balance, tissues of the host obviously supply nutrient substance for continued riotous proliferation.

Another fundamental observation is that spontaneous and induced tumors appear later and in a smaller percentage of animals when the total caloric intake is restricted. Thus,

spontaneous mammary carcinomata, subcutaneous tumors produced by injection of a carcinogen (such as methylcholanthrene), skin tumors resulting from the painting of a carcinogen or from ultraviolet radiation, induced and spontaneous lung tumors, and spontaneous and induced leukemias are all influenced by caloric limitation. The reduction in the average appearance time and in the incidence of such tumors is, in general, proportional to the degree of caloric restriction. In fact, the effect of caloric intake on carcinogenesis is so pronounced that this influence must be considered in interpreting any experiment involving the influence of secondary factors on tumor formation. Thus, the retardation of tumor incidence and growth by some agent may well be the indirect influence of reduced caloric intake resulting from treatment rather than the direct consequence of specific therapy.

Liver Tumors

While formation of experimental tumors is generally decreased by procedures leading to malnutrition, the findings for induced liver tumors are somewhat different in that malnutrition (resulting chiefly from dietary restrictions) tend to enhance rather than inhibit carcinogenesis. As previously indicated, the prime requisite for production of liver cancer in rats by azo dyes is a relatively low protein diet; rice flour, containing about 9% protein, is usually used as the basic diet. Production of liver tumors can be retarded by addition to the basic diet of small amounts of liver (Nakahara, *et al.*, 1938), liver extract, kidney, yeast, rice bran, rye (Sugiura, *et al.*, 1940), riboflavin, casein (Kensler, *et al.*, 1941), milk (Sugiura, 1944), egg albumin, hydrogenated cocoanut oil (Miller, *et al.*, 1944), and adsorbents (Hanszen and Selle, 1943). On the other hand, liver cancer is accelerated by the addition of cholesterol, lanolin, phosphorus and arsenic. A pro-carcinogenic action has been reported for biotin (du Vigneaud, *et al.*, 1942; Burk and Wenzler, 1944), pyridoxine (Miner, 1943), and fats (Miller, *et al.*, 1941, 1944-a). Various views have been advanced to account for the effect of these dietary constituents (Rusch, *et al.*, 1945). Some workers think the action specific, others non-specific. However, since the fundamental mechanism of cancer is still

not known, the manner by which diet influences tumor formation is obviously not understood.

This laboratory has found that for certain diets containing moderately high protein levels, the substitution of sugar for fat tends to diminish the incidence of liver tumors. For protein levels below 12%, the substitution of sugar has no beneficial effect upon the high tumor incidence obtained with high fat diets. Caution must always be used in evaluating effects of dietary constituents, particularly in rats since these are the only animals so far found which develop a high percentage of induced, primary liver tumors. Any attempt, therefore, to interpret the foregoing facts with our present state of knowledge is not justified. Experiments are in progress now to confirm the observations in monkeys.

Diet and Liver Cancer in Man

Dietary studies on tumor production in experimental animals take on an added interest as a result of the finding that certain factors influencing tumor formation in animals also appear to promote the formation of tumors in man. The prevalence of primary cancer of the liver in certain parts of Asia and Africa is a problem that is attracting increasing attention. It is well known that rice is the predominant constituent of Oriental diets, and that the incidence of primary cancer of the liver of Orientals is much higher than is that of Americans and Europeans, usually stated to be less than 2%. According to Rusch, *et al.* (1945), primary liver cancer ranks third in frequency of all cancer cases, both in males and in females, in Japan. In China, hepatic cancer ranks fifth in males and eighth in females, while in Korea it ranks third in males and fourth in females. The incidence of primary liver cancer of Orientals, according the Hoch-Ligeti (1946) is 15% to 36%.

A further example of human dietary experiences paralleling animal experimentation is the high cancer mortality among the colored people in Africa who subsist on greatly restricted diets. Berman (1935) found among the native male population of South Africa an incidence of 32% for primary carcinoma of the liver; in mining areas around Johannesburg the incidence was said to be 90.5%.

Later data collected by Berman (1940, 1941) indicate that primary cancer of the liver is prevalent in varying degrees over the southern half of Africa, India, the East Indies, China, and Japan. His figures for various races, listed in order of frequency of primary hepatic carcinoma are:

Race	*Per cent*	*Race*	*Per cent*
Bantu	37.0	Philippines	22.0
Javanese	36.0	Semi Bantu	18.7
Chinese	36.0	Japanese	7.8
Asiatic Indians	28.0		

While parasitic conditions (helminthiasis, malaria, and schistosomiasis) have been suggested as the most important factor in the high incidence of liver cancer in these people, and also in Orientals, certain writers (Rusch, *et al.*, 1945; Kennaway, 1944; Gilbert and Gillman, 1944; and Hoch-Ligeti, 1946) believe that experimental evidence points to diet as a major contributing factor. Hoch-Ligeti points out that over the vast Asiatic and African areas mentioned above, there is, of course, a great variety of dietetic conditions and habits, about which it is difficult to collect complete information and thus it is impossible to generalize. Nevertheless, it seems unlikely that any single positive factor, that is, a common, ingested carcinogenic substance, operates over so many different countries, though such a factor may be present in some areas. In this connection, Hoch-Ligeti (1946) has suggested that a deficiency in the diet is more likely to be common to all these peoples.

Gilbert and Gillman (1944) mention that cirrhosis of the liver is found in 80% of the autopsies performed on male Bantus at the Johannesburg General Hospital. This they suggest is probably related to the dietary intake, since, because of great poverty, the natives are forced to live almost exclusively on the cheapest available food (corn). The protein level is extremely low; meat is a treat and is eaten only occasionally, at special ceremonies or when cattle or sheep die from disease or starvation. It is well known, of course, that deficiency diseases of all kinds, including pellagra, are widespread among the black people of South Africa (Gilbert

and Gillman, 1944). Nor is primary cancer of the liver peculiar to the colored race. Kennaway (1944) points out that in America this condition is as common among whites as Negroes. Actually, Kennaway (1944) found more cases among the general population in such states as Maine and Idaho as in Alabama or South Carolina.

To determine the effect of the drastic South African diet on the incidence of cancer, Gillman, *et al.* (1945), subjected rats to the main constituents of this (Bantu) diet. Within slightly more than a year most of the rats lost their hair and developed liver lesions which varied from fatty changes to extensive cirrhosis. Marked cirrhosis was found in 20% of the animals. A similarity between the condition of the experimental animals and that of humans on the severely deficient diet is too striking to be ignored. It is quite possible, therefore, that in liver cancer in man carcinogenic agents may be concerned, the manifestations of which are modified by dietary factors.

Summary

Obviously, the ultimate aim of cancer investigations such as those related is a better understanding of carcinogenesis. Studies on experimental liver tumors, particularly those concerning diet, seem to raise more questions than they answer. However, continued work of this type may lead to the discovery of inhibiting factors feasible of human application. We all look and work toward that day when this is possible.

In the pathogenesis of liver tumors in man, two factors are worthy of consideration: long persisting and restricted dietary habits and metabolic carcinogens.

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RHEUMATIC FEVER*

ARILD E. HANSEN

Rheumatic fever may be considered to be one of our chief medical problems. It is one of the main causes of death from disease in the school-age child. Almost any year, when we study mortality data in the five- to fourteen-year age group, we learn that this condition is responsible for more deaths than is diphtheria, poliomyelitis, scarlet fever, typhoid fever, measles, mumps, and meningococcic meningitis combined. Hence, even on the basis of mortality alone, this disorder is an important problem. Besides, we have no true index to the extent of its ravages in producing disability and crippling—"cripples who do not limp" or "cripples who cannot even walk."

In contrast with previous years, there has recently been an acute awareness of this problem not only medically but socially and economically as well. We ask, and quite naturally so, "Why is this?" Is the disorder increasing in frequency or is it a matter of relatively greater importance or are both we as physicians and the public itself gradually awakening to its real significance? Were our pioneer colleagues confronted with this same problem? Were they ahead of us or were they lacking in true appreciation? It is my belief that it is the nature of the disease itself which has made it a difficult problem. Thus, attention to it has been retarded. Why is the disease so serious? Certainly, it is not on the basis of the disconcerting pains in the joints so often fleeting in nature and consequently considered innocuous, or even the unsocial, involuntary body gyrations of the child with St. Vitus dance. It is that no matter what the symptomatology may be, the heart is being damaged. Our great writers of the past seem to have overlooked this strange association—the varied symptomatology and cardiac damage.

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*Presented at the Sixth Annual Graduate Seminar Alumni Association of the Medical College of the University of South Carolina, Charleston, South Carolina, November 5, 1947. From the Department of Pediatrics and Child Health Program of The University of Texas Medical Branch, Galveston.

It is a privilege to speak in Charleston where lived the first person, as Major emphasizes, to point out the remarkable association of rheumatic joints and heart disease. Doctor William Charles Wells was born in Charleston in 1757. After starting his studies with Doctor Alexander Garden, the leading physician in Charleston at that time, he continued his medical training in England. In 1810, he published an account of the cardiac complications of rheumatic fever. He had observed two patients, a young man of eighteen and a girl nearly fifteen years of age. According to Wells, the young man had his first attack of acute rheumatism at the age of nine. Four subsequent attacks were severe enough to confine him to bed for several weeks. Later, the pain shifted and he had a sense of oppression in his chest. Four weeks before seeing Doctor Wells, he had fallen into a pool and had allowed his soaked clothing to dry on him. The following day, he felt cold, his legs bothered him, and he had the feeling of faintness, nausea, chest oppression followed by palpitation of the heart and a "beating" in the head. The patient then suffered from breathlessness, choking and a feeling as if he were about to expire. Doctor Wells seemed to place some significance on the fact that an uncle of the patient had been known to have similar type of history and at autopsy had been found to have an enlargement of the heart. Doctor Wells also cited the occurrence of numerous small tumors along the tendons of the fifteen-year-old girl who had rheumatism and suffered "violent beating of the heart." Thus, the first detailed account was reported by Doctor Wells, although in introducing his paper, Wells commented that Doctor David Pitcairn in 1788, remarked that persons subject to rheumatism were attacked more frequently than others with symptoms of organic disease of the heart. Approximately 32 years after Doctor Wells' death in 1817, Doctor Elisha Bartlett delivered an address to the Louisville Medical Society, "A Brief Sketch of the Life, Character and Writings of William Charles Wells." In this he stated, "And I take up this subject, not as a piece of irksome drudgery, but as a labor of love. I had just finished reading, when I prepared this sketch, for the first time, the volume which contains the record of his life and the results of his

labors; and my mind has been so interested and my feelings so warmed by its perusal, that the business before me became a pleasure, and not a task. I cannot doubt that I shall awaken in your bosoms emotions and sentiments corresponding in some degree to those which have kindled in mine." That quotation is the essence of the admiration given Doctor Wells.

No matter how many may be the problems of rheumatic fever and rheumatic heart disease, there are two which stand out, namely, diagnosis and management. We are ever concerned with scientific endeavors and many attempts have been and are being made to learn more about the fundamental nature of the disease. Interest in a disease is not necessarily proportionate to the money and effort expended in its behalf. Most of us could not help but be impressed with the statement which received quite widespread publicity last year and pointed out that for each death from poliomyelitis, some several hundred dollars were spent in research work; for each death from cancer, a couple of dollars, and for each death from heart disease, just a few cents.

In the etiology of rheumatic fever evidence is accumulating to show that hemolytic streptococci Group A is involved. Likewise, considerable evidence has been gathered to indicate hereditary features. Wilson finds this to be a recessive gene pattern. Attempts have been made to implicate viruses, even rickettsiae, as well as other bacteria. The phenomenon of allergy has been called forth to explain the pathological picture in this disease. In fact, the expression "hypersensitivity" is used quite frequently to account for the microscopic vascular and collagen changes in the tissues of patients with rheumatic fever. Cavelti, by auto-antibody production in experimental animals by injection of tissue extracts together with hemolytic streptococci as the antigen, revealed there are histologic features in the tissues which are similar to those found in acute rheumatic fever. Much is yet to be learned about the mechanisms involved in producing the well described and defined pathology of rheumatic fever. Nevertheless, from a practical viewpoint, it seems now to be quite well agreed that the following pattern is followed:

1. Initiating throat infection with hemolytic streptococci, Group A. This is usually of 1 to 3 days duration.
2. The latent period of one to three weeks duration. Army experience disclosed this to be 16 days on the average.
3. Period of rheumatic activity of variable degrees of severity and duration.
4. Quiescent period of different lengths with strong likelihood of development of a recrudescence.

Diagnosis:

There is no simple method by which the diagnosis of acute rheumatic heart disease may be made. It is possible to do so only on clinical grounds. No short cut laboratory tests can serve as a substitute for good clinical practice. Hence, it is necessary to have a complete and accurate history, thorough physical examination, certain laboratory data and the ever important feature, namely, the intimate knowledge of the basic aspects of the disease itself. In some instances, the diagnosis of acute rheumatic fever is made very easily because of the typical migratory joint involvement or possibly, the presence of outspoken chorea-minor. In other instances, the diagnosis may be very difficult. At times, in dealing with students, we find it helpful in attempting to diagnose a given case to list the various features from the history, physical and laboratory examinations and to mark in columns + or — those features which favor the diagnosis of rheumatic fever. By so doing, often it becomes apparent that one is or is not dealing with rheumatic fever. The author in 1944 presented data dealing with the diagnosis of rheumatic fever from the viewpoint of differential diagnosis.

We may now turn to the subject of treatment of acute rheumatic fever. This will be discussed in relation to the acute, subsiding, convalescent, and quiescent phases of the disease. For most satisfactory management of the particular child, it is necessary to know whether or not the infection has subsided or if there is still activity of the process. For this, obtain all the features from the history; evaluate all data from the physical examination, particularly with reference to the heart;

and in addition, consider the results of the various laboratory tests. Again in this evaluation all possible objective bits of information must be utilized.

Management of the Acute Phase:

Rest in bed. It is very important that the child who has active rheumatic infection be kept quiet in bed. There is no rule as to how long—each case unto itself. Mild sedation such as phenobarbital may be employed to assist if necessary. Attempt to provide good food.

Relief of pain and fever. Particularly helpful is the symptomatic therapy with salicylates. Sodium salicylate (grain per pound or 100–150 mg. per kilogram body weight) administered in four to six daily doses is very helpful. Aspirin may be used very satisfactorily. One word of caution perhaps should be mentioned in regard to the likelihood of salicylism developing when sodium salicylate is employed or when salicylates are used in large amounts as advocated by some. The development of respiratory stimulation of central origin with subsequent development of acidosis may cause a serious condition or even fatality from salicylates. One should be cautious whenever this drug is used with children.

Chorea. Bed rest, freedom from external stimuli, sedation with phenobarbital are quite satisfactory in most cases.

Congestive failure. Complete rest in bed, oxygen tent, sedation, even employing narcotics if necessary. Use of simple diuretics, or occasionally combinations of mercury and aminophylline may be helpful. Many different opinions are expressed regarding the use of digitalis for the child who is suffering from congestive failure. In many instances, it appears that the employment of digitalis preparations seems futile. At times, however, good results are obtained. Because of the danger of intoxication from digitalis, it is well to check the dosage with frequent electrocardiograms.

Management in the Subsiding Phase:

The feature which characterizes the subsiding phase is that most of the children are remarkably comfortable. Yet, one knows the children should be given the benefit of bed rest. The primary objective during this phase is to continue rest

in bed. The optimum duration of this period in order that the child may have the utmost benefit and yet remain satisfied is difficult to determine. Each case must be individualized, but certainly the safest rule to follow is to wait until all signs of rheumatic infection have disappeared before the patient gradually is allowed to resume normal physical activity. The use of "stay-in-bed" clubs for exchange of books, toys and games may help to keep these children happy. Occupational and play therapy are very important, but emphasis should be on such occupational therapy as can be done in bed without demanding physical exertion or causing excitement. Such devices as mechanical supports for books and suspension of toys from frames above the bed will relieve physical strain while the child is being entertained. When the rheumatic fever activity has subsided, gradual resumption of physical exertion may be instituted in accordance with the routines usually prescribed. During this stage, we should pay especial attention to supplying the right diet with vitamin supplements and iron. Occasionally it is necessary to give small blood transfusions to correct the tendency towards anemia.

Convalescent Care:

Actually to define convalescence is rather difficult, but we might assume this begins when all signs of rheumatic infection have disappeared and ends when recovery is complete and full resumption of physical activity takes place. Strictly speaking, we should consider this to be until undisputed quiescence has been obtained. This period is indeed variable. In some instances this is one to two months and in others, one to two years. As an average, we should think of it as four to six months.

On this occasion, I do not feel we should debate the merits of individual home convalescence in contrast with well-supervised convalescent homes. The communal and social aspects of the rheumatic fever problem are receiving the much needed attention in many localities, but it is sadly neglected in others. In most instances the individual physician must direct the care for the individual child. It should be borne in mind that more disastrous results occur by far because

of reactivation of the rheumatic infection than result from excessive physical activity. Nevertheless, caution should be the rule, and it is better to advise a few weeks of extra quiet than to allow strenuous activity too early. In principle, it is better to state in a positive manner the types, kinds and times of activity allowed rather than to list limitations and "do nots." A routine should be planned for gradual resumption of physical activity, *e.g.*, feeding self, games in bed, sitting up in bed, sitting in chair, bathroom privileges, meals with family, walking inside, walking outside, school work at home, mild play activities, part day in school, morning and afternoon rest periods. The introduction of each new type of activity should be separated from one half to one week in those patients with little or no evidence of carditis and from one to two, or even three weeks where there has been cardiac involvement.

Management During Quiescent Phase:

The key feature or motive of the quiescent phase is the prevention of recurrences. Removal of tonsils and adenoids is not helpful. We will dismiss the questions of climate, diet, biological methods of prevention, use of salicylates and confine our remarks to the use of sulfonamides in the prevention of rheumatic recrudescences. For almost a decade now, sulfa compounds have been used for this purpose. In most instances the results have been considered gratifying. It appears that the chance of a child who is receiving sulfa compounds continuously of developing a flare-up of rheumatic infection is one-tenth that of the child who does not. Major recrudescences are indeed unusual when patients are on this regime.

The following outline may be helpful:

(1) There should be no evidence of activity of the infection.

(2) Type of sulfonamide compound.—Most of the studies have been with sulfanilamide. However, sulfadiazine and sulfamerazine should be fully as, or probably more, satisfactory because of the smaller dosage required and the lesser toxic effects.

(3) Dosage.—Most workers now agree that blood sulfonamide levels between two and three mg. per cent appear adequate for prophylactic purposes. To produce these levels, sulfanilamide 0.6 gm. with the morning and

evening meals is advised for the older children or adults, while 0.3 gm. three times daily is sufficient for smaller children. If sulfadiazine or sulfamerazine is used, either 1.0 gm. once daily or 0.5 gm. twice daily is given to older children, with 0.5 gm. once daily for the younger subjects. In initiating therapy, one-half of the maintenance dose is suggested for the first week.

(4) Precautions.—Watch for signs of toxicity or idiosyncrasy such as skin rashes, gastro-intestinal upsets, hematuria, depression of hemoglobin, leukopenia, and agranulocytosis; fortunately, these are seldom severe enough to require cessation of the drug.

(5) Blood level of sulfonamides.—It is desirable to determine this early in the course of therapy and at regular intervals subsequently to determine the adequacy of the dosage. This procedure also may serve as a check on the coöperation of the patient in taking the drug.

(6) Evaluation of patient.—Routine physical and laboratory examinations every four to six weeks are necessary in order that the physician be aware constantly of the patient's status. One should be alert to evidences of activity of the rheumatic infection.

Summary

The problem of rheumatic fever is one of the most important in the school-age child. Every physician who sees children in his practice must be familiar with the problem as he must assume the greatest responsibility in the solution of the problem. It is only the physician who is equipped to make the diagnosis as this is made entirely on clinical grounds. Proper management demands an understanding of the significance of rest, diet, symptomatic treatment and the emotional and educational factors. The patient should be under surveillance for a period of years. The control of recrudescences is a distinct advancement in regard to conquering the problem.

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EFFECTS OF "BENZEDRINE"* SULFATE ON ESTROUS CYCLES OF RATS†

J. Eugene Hill, Paul L. Ewing, and G. A. Emerson

As a part of a study of toxicity of dl-2-amino-1-phenylpropane ("Benzedrine," Amphetamine Sulfate N. N. R.) and related agents, it was of interest to test non-lethal toxicity by some quantitative measure. Long and Evans (1922) state that interference with the estrous cycles of white rats constitutes a more sensitive indication of well-being than the usual criteria of changes in body weight, condition of the fur, or other common objective findings. Effects of "Benzedrine" on estrous are of further import in regard to the use of this agent in treating such conditions as menopausal disturbances, obesity, anhedonia, alcoholic psychoses and involutional melancholia.

Inanition, *per se*, strongly inhibits estrus in the rat (Mulinos, *et al.*, 1939); estrus-inhibiting effects of bilateral adrenalectomy, dietary deficiencies including athiaminosis, and treatment with dinitrophenol or thyroid extract are attributed at least in part to inanition. Treatment of rats with insulin, protamine insulin or protamine alone (Williams, 1938), morphine (Myers, 1931), or nicotine (Behrend and Thienes, 1932) does not result in inanition or inhibition of estrus. In rabbits, electrical stimulation of the brain or intravenous injection of picrotoxin is known to produce ovulation and consequent changes in estrus (Marshall, *et al.*, 1939), although many other stimulants such as pilocarpine, physostigmine, acetylcholine, epinephrine, strychnine, apomorphine, tetrahydronaphthylamine, ergonovine, methacholine, and coriamyrtin, do not share this action. Brooks, *et al.* (1940), also note the inefficacy of strychnine, insulin, "Benzedrine," and ephedrine in initiating ovulation in the rabbit. In rats, electrical stimulation of the head is 68% effective in inducing pseudopregnancy (Harris, 1936).

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*Racemic Amphetamine Sulfate, S. K. F.

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Intravenous injection of copper acetate, picrotoxin or metrazol induces ovulation in rabbits only if the hypophyseal stalk is intact (Brooks, *et al.*, 1940). All of these actions are presumably mediated through the central nervous system. Taubenhaus and Soskin (1941) note the effects on function of the rabbit pituitary by substances from the hypothalamus presumed to be acetylcholine-like since atropinization of the pituitary blocks them. With "Benzedrine," there is a possibility of effects on estrus both through derangement of nutrition, with large doses, and through excitant actions on the hypothalamus or other parts of the central nervous system activating the pituitary.

Experimental

Four types of tests were made: *A.*, with massive repeated doses of "Benzedrine" sulfate in thirteen rats housed together; *B.*, with even larger doses repeated in a small group; *C.*, with a single small dose in twenty rats with irregular cycles; and *D.*, with single large doses, within the lethal range, of "Benzedrine," *l*- and *d*-amphetamines, and *d*-desoxyephedrine ("Pervitin").[1]

In comparing effects on the estrous cycle, it was considered sufficient to use as a basis for calculation the total number of estrous cycles within a defined period, counting any interval of estrus as one cycle. Thus, if the first vaginal smear examination revealed that an animal was in Stage III of a cycle, and the last within the period showed Stage II, both the first and last intervals of estrus were counted as complete cycles. This introduced an error, particularly for small groups in which there might be less chance of compensation, *e.g.*, by animals in Stage V at the beginning and end of a period of observation. However, it was felt that this error is below that which might obscure any marked effects of "Benzedrine," and further, that since observations were made only at twenty-four hour intervals, the data were not susceptible of more accurate treatment. Accordingly, values given below are based on this

[1]Trade-mark name of Smith, Kline & French Laboratories for *d*-desoxyephedrine.

method of calculating the frequency of estrous cycles; where appropriate, the standard deviation of the mean is also given.

A. *Effect of repeated large doses of "Benzedrine" sulfate.*

Thirteen adult female white rats, 182–264 grams in weight (average 218 grams) were treated daily for ten days with 15 mg./kg. of "Benzedrine" sulfate intraperitoneally, a total of 150 mg./kg. Estrous cycles had been followed by daily examination of vaginal smears for twenty-one days before treatment, and observation was continued daily during and after treatment. Five days after conclusion of this treatment, the thirteen rats were treated twice daily for four days with 25 mg./kg. of "Benzedrine" sulfate, a total of 200 mg./kg. Three rats died during this treatment; vaginal smears of the remaining ten rats were examined daily for fifty-seven days after this second treatment was concluded.

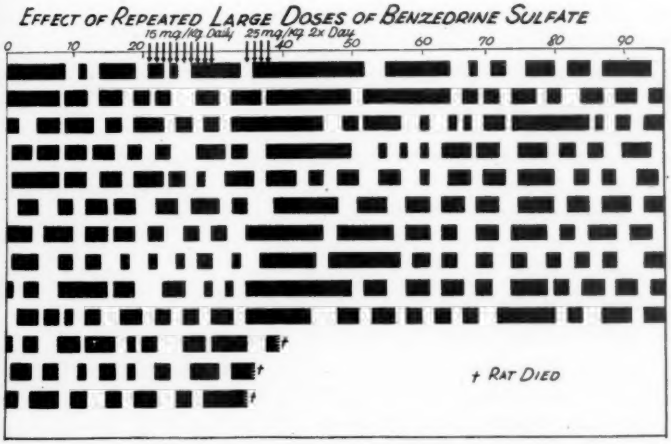

FIG. 1. Estrous cycles of 13 rats before and after repeated intraperitoneal injection of "Benzedrine" sulfate. Di-estrus is represented by black bars.

Effects of these treatments on the estrous cycles of individual rats are presented graphically in Figure 1; shaded areas represent periods of di-estrus and white areas represent periods

of pro-estrus and estrus. The major effect observed is a lengthening of the periods of di-estrus following the second course of intensive treatment.

The mean period/cycle for the thirteen rats during the initial twenty-one day pre-treatment period was 4.63 days. For the next 14 days, including the ten days of treatment with 15 mg./kg. of "Benzedrine," the mean was 4.55 days per cycle. Omitting the period of more intensive treatment, the mean for the twenty-one days following this second period of treatment was 8.75 days/cycle, for the ten survivors; for the first ten days of this post-treatment period, 14.3 days/cycle. Finally, the mean for the last 36 days, starting twenty-one days after the last treatment, was 5.14 days/cycle.

The above figures denote the length of the estrous cycle in the "average rat." They are obtained by dividing the total number of cycles for the whole group by the number of rats and the number of days in the observation period. At the suggestion of Dr. J. Allen Scott, we have also calculated the length of the average cycle by averaging the means of the number of days/cycle for the individual rats. This calculation gives somewhat different information and emphasizes the influence of irregular cycles.

The length of the average cycle, so calculated, was 4.81 ± 0.33 days for the pre-treatment period of twenty-one days; 4.77 ± 0.31 days for the next fourteen days including the period of treatment with the lower dose of "Benzedrine"; 9.08 ± 0.82 days for the twenty-one day period following treatment with the larger dose of "Benzedrine"; and 5.19 ± 0.20 days for the final thirty-six day period.

B. *Effect of repeated large doses in a small group.*

The thirteen rats described above were housed together in a large cage. It is known (Chance, 1946; Ewing, *et al.*, 1947) that lethal toxicity of "Benzedrine" is decreased by segregation of rats in separate cages. It was therefore of interest to test the non-lethal toxic effects of "Benzedrine" on a small group.

Three rats housed together in a large cage, approximately 100 sq. in. per rat or 1800 cu. in./rat, were treated for four days with 25 mg./kg. of "Benzedrine" sulfate intraperitoneally, a total of 100 mg./kg. Five days later the same rats were

treated twice daily for four days with 37.5 mg./kg. of "Benzedrine" sulfate, a total of 300 mg./kg. Daily vaginal smears were examined for forty-six days before the first treatment, during the treatment periods, and for thirty-six days following the last treatment. One rat died during the second treatment period, and another died of pneumonitis twenty-one days following the last treatment. Estrous cycles of the three rats are noted in Figure 2.

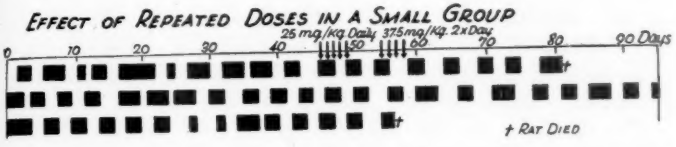

FIG. 2. Estrous cycles of 3 rats before and after repeated intraperitoneal injection of massive doses of "Benzedrine" sulfate.

For the forty-six day pre-treatment period, the mean period/cycle was 4.18 days. Fortuitously, this value was the same for each of the three rats. For the periods including treatment and subsequent survival, the first rat showed a mean period/cycle of 5.0 days, for thirty-five days; the second, 4.46 days, for forty-nine days; and the third, 5.5 days, for eleven days.

C. *Effect of a single small dose.*

During and after the first treatment period in the group of thirteen rats noted above, there was a small decrease in the length of the average cycle, although this was not significant. There appeared also to be a tendency for the pro-estrous and estrous portion of individual cycles to increase occasionally, relative to the di-estrous portion; this tendency may also be noted in the group of three rats treated with repeated massive doses of "Benzedrine." None of this may be considered conclusive in suggesting an estrus-favoring action of "Benzedrine," but it was felt that this point should be studied with a single small dose.

Vaginal smears were examined daily for fifty-one days in a group of twenty rats chosen for irregular cycles. The usual precautions and care in handling were strictly followed, so that pseudopregnancy was no more likely in this group than

in the others reported here; also, among some 150 rats in which daily vaginal smears have been made in this laboratory, no instance of induction of pseudopregnancy due to this procedure was noted.

FIG. 3. Estrous cycles of 20 rats treated intraperitoneally with a single dose of "Benzedrine" sulfate.

The mean period/cycle for these twenty rats was 6.30 days, over the fifty-one day period. All twenty were then given 5 mg./kg. "Benzedrine" sulfate intraperitoneally, and daily vaginal smear examinations were made for sixteen days. The mean period/cycle during the post-treatment interval was 4.85 days.

The length of the average cycle in these rats during the pre-treatment period was 7.24 ± 1.00 days; during the post-treatment period, 5.05 ± 0.20 days. The probability of this

difference occurring through chance is approximately 0.03. All of the rats but one had a smaller maximum period of di-estrus after treatment than before. Individual cycles are noted in Figure 3.

D. *Effect of a single large dose.*

Vaginal smears of fifteen rats surviving oral doses of "Benzedrine" sulfate, *d*-amphetamine sulfate, *l*-amphetamine sulfate, and *d*-desoxyephedrine hydrochloride within the lethal range (60–80, 45–60, 150–200, and 35–50 mg./kg., resp.) were examined daily for twenty days. The mean period/cycle for the first ten days after treatment was 4.29 days, and, for the entire twenty days, 4.62 days. The length of the average cycle for the ten- and twenty-day periods following treatment was 5.03 ± 0.45 and 4.98 ± 0.69 days, respectively. Two of five rats treated with "Benzedrine" showed periods of di-estrus of eleven and twelve days, respectively. The longest period of di-estrus in four rats treated with *d*-amphetamine was four days; with *l*-amphetamine, one of four rats had periods of di-estrus of five and six days, while the three others had normal two- to three-day periods of di-estrus. Each of two rats treated with *d*-desoxyephedrine showed prolonged di-estrus, of thirteen and fourteen days, respectively. Apparent differences between agents cannot be considered significant without further study.

E. *Comparison with normal rats.*

In all the above tests except the last, preliminary observation periods serve as controls for the drug effects. A comparison of these control periods with similar intervals observed in untreated rats maintained under similar conditions may also be made.

In a group of twenty-six rats not undergoing treatment, the mean period per cycle for a twenty-five-day interval was 4.92 days. Another group of twenty-one rats showed a mean period/cycle of 4.25 days, over a twenty-day interval. The length of the average cycle in these two groups was 5.08 ± 0.19 and 4.30 ± 0.12 days, respectively. These values agree with

those in the literature, and with all preliminary control observations in the treated rats except for that of the group of twenty rats chosen for their irregularity.

Discussion

Large but sublethal doses of "Benzedrine" have little effect on the regularity of the estrous cycle, while doses within the lethal range markedly prolong the subsequent periods of di-estrus in the survivors. *d*-Desoxyephedrine, in two rats, caused prolonged di-estrus, while the optically active isomers of "Benzedrine" do not appear to disturb the estrous rhythm. The possibility that a potentiation of non-lethal toxic effects of "Benzedrine" occurs when rats are grouped together, similar to the marked effects of segregation on the lethal toxicity, is suggested by the lack of disturbance of regular cycles in three rats given even larger doses but housed under conditions in which they had more space than rats in the larger group.

A single dose of 5 mg./kg. appeared to shorten subsequent estrous cycles in twenty rats with irregular cycles before treatment; the difference, though large, is not statistically significant. This dose is much greater than the equivalent therapeutic dose for man, and is sufficient to disorient rats to the extent that they are unable to run through a maze (Ewing, *et al.*, 1947). Since there is no apparent lengthening of di-estrus with this dose, further studies were not made at this or lower doses.

The estrus-inhibiting effect of 25 mg./kg. of "Benzedrine," repeated twice daily for four days, is accompanied by inanition, reflected in a 10% loss of weight within the four days. However, effects on estrus do not parallel the rate at which individual rats regained their weight after treatment. The role of inanition in the effect of "Benzedrine" on estrus thus is questionable.

Summary

Estrous cycles of fifty-one rats were noted after treatment with "Benzedrine" sulfate (racemic amphetamine sulfate, S. K. F.) or related agents. Effects of "Benzedrine" were estimated by comparison of the regularity of cycles after treat-

ment with those during the pre-treatment period in the same rats and with those of forty-seven untreated rats. Only in a group of thirteen rats housed together were significant effects obtained with doses of "Benzedrine" within the lethal range, with definite prolongation of di-estrus. On treatment with a single dose of 5 mg./kg. of "Benzedrine" sulfate, a group of twenty rats with previously irregular cycles showed a questionable stimulant effect on estrus; this was not statistically significant ($p = 0.03$). Factors of inanition and of hypothalamic stimulation are considered in relation to effects on the estrous cycle.

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PLACENTAL-FETAL WEIGHT RATIOS*

JOHN G. SINCLAIR

The placenta is a living filter between the maternal and fetal blood streams. The microscopic character of the villous surface determines the qualitative selection of the transudate and the amount passed per unit area. The total area determines the total flow of the exchange and therefore places limitations upon the maximum attainable size of the fetus. There is, during most of the gestational cycle, a large reserve above metabolic needs, but at birth that reserve is encroached upon until it becomes critical.

Many attempts have been made to determine the quantitative relations between birth weights and placental weights, volumes or areas. These have not been highly successful. Christofersen (1941) calculated that a villous area in mm^2 equals .80 of the volume in $mm^3 \times 33.6$. He found in perfusion experiments that the fresh placenta was capable of passing the known production of CO_2 from the fetus and he devised a satisfactory formula based on calculated areas and simple diffusion principles. He assumed a fixed ratio of placenta to birth weight. The difficulties of measurement have been due to such variables as blood clots, total content of maternal blood, non placental chorion, amnion, decidua and cord. Part of the difficulties lie in errors of weighing, converting and recording both birth and placental weights because the work is done by many individuals, most of whom are in a hurry. We have noted consistently more deliveries on week-ends, as though some psychological or extraneous physical factor precipitated birth. A great deal of variation in placental weights lies in the amount of retained fetal blood when the cord is prematurely clamped.

Mackness in 1889 thought he found larger placentas in older women but otherwise saw no correlation. (Smith (1891),

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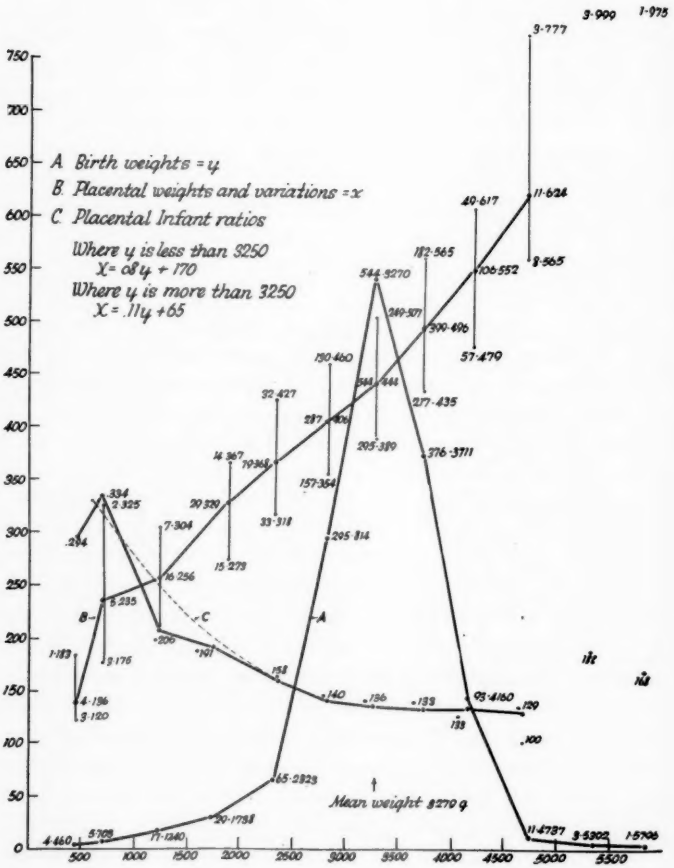
Zentler (1891), Spameni (1901), Laurent (1913), and Holland (1922), quoted by Adair (1925), all noted that large babies had large placentas. Adair and Thelander (1925) studied 392 placentas, separating out 18 premature cases. They stripped the membranes, weighed the placentas and determined the superficial area. The data are grouped as to sex. The average weight at birth was 3,368 grams and the average placental weight was 473 gms., a ratio of 1:7.27 with a correlation coefficient of about .63. Harer (1936) says that he weighed and measured 1,000 placentas but he does not utilize the data on weight in his article. Wolfram (1941) has not been available. The title of his paper indicates a doubt that there is any close correlation between these variables. Dow and Torpin (1939) made an intensive mathematical study of a small group (269) babies, including their weights, placental weights, superficial areas and gestation sac size. Correlations between two, three and all four variables are plotted. The results of this study are quite different from the present one and not easily comparable.

Knowing the inherent errors of weighing such material, and confident, from studies to be published later, that we had eliminated a number of highly independent variables in the cord, chorion and amnion, it is felt that the data do not deserve more statistical treatment than is being given to it here. The data have been tabulated at three intervals; after 500, 1,000, and finally the present nearly 1,500 specimens. It is only after the latter number that the distribution curves and ratios smoothed sufficiently to appear satisfactory. Even then the interpretation of extreme values is on questionable ground for lack of sufficient numbers. Sometimes the calculation of correlation coefficients gives a false sense of accuracy.

Some of the studies in the group to be reported were begun in 1931 but the bulk of the collections were made from 1939–41, using all the placentas delivered at two local hospitals. Sinclair (1942) gives a short summary of the work at that time. We have tried to trace and check any aberrant values at the time they appeared. We carefully remove all extraneous membranes and clip the cord close to the surface.

Observations are made as to cord position, thickness and discoloration. Chorionic color, decidual thickness and calcification and any unusual features of form or thickness of the placenta are recorded. The whole birth record is abstracted.

At this time we report weights and ratios of infants and placentas from 1,443 live births. We shall treat stillborns later. We have also set aside all multiple births. The problem of



prematurity is intriguing. Clinicians set an arbitrary value of 2,500 gm. for white babies and 2,250 gm. for colored babies below which all babies are called premature. There is no logic to this rule except that of playing safe. All prematures (or immatures) are included along with many small but full term and fully differentiated babies. For our purposes this is a mixed group. With much the same logic we have included all excessively large babies among which are the postmatures.

The distribution of birth weights, Graph A, is very normal. We divided the material into groups differing by 500 gm. which gave us many classes, each containing large numbers. Our mean value comes between Arey's 3,254 gm. and Adair's 3,368 gm., falling at 3,279 gm. Note that there are probably many more premature than postmature individuals. This probably causes a shift of the mean value a little to the left of its true value for full term births. Note that we have used the actual mean values of each sub-group and not the median values.

The graph of placental weights (B, Fig. 1), plotted at 0.1 the value of the fetal weights, forms a smooth sinusoidal curve in which every point except extremes lies on the line. It is observed that the mean point of the curve is the point of inflexion. Treating this curve as straight lines with different trends above and below the mean value the bulk of the observations can be reduced to two linear formulae. Placental weight below value equals 170 plus, 0.08 times the birth weight in grams. Placental weights above mean value equal 65 plus, 0.11 of birth weight in grams. These are, of course, approximations to calculate average weights. Deviations from these values are shown as short vertical lines across the placental curve. The length of the lines is the average deviation above and below mean values. Note that the deviations, while fairly broad, are very regular, the lower value forming a curve as smooth as the mean curve. The number of individuals showing minus deviations is always greater than the number above the line, indicating that the placenta may be much larger than necessary at birth but it cannot be much smaller.

The placental curve shows a definite trend when related to birth weight. This is best illustrated in the graph of ratios (C) plotted by using 0.01 of the values on the vertical column against birth weights. It is noted here that within the limits of the major portion of the distribution the ratios fall continuously from 0.158 to 0.129, the latter figure indicating the lowest average value compatible with intrauterine life. If the extreme deviation at the low point be taken at twice the average value we arrive at the probable absolute minimum of 0.10–0.11 of which possibly 100 gm. is nonfunctional tissue which cannot be separated out.

The data presented are in keeping with the concept that the placental barrier approaches a limit of transfer, qualitatively and quantitatively, at the time of gestation, that expresses itself in anoxia if either of two conditions prevail. These are: a sudden increase in oxygen demand by the fetus because of activity, or any condition interfering with the supply of blood to maternal sinuses. It is known from the observations of Markee (1940) that uterine vessels show rhythmic activity which because of periodic variations in severity go beyond transient anemia to become the incipient cause of menstruation. We also know from Walther Schmitt (1929) that the same type of rhythmic activity is seen in the placental vessels. Snyder (1938) regards parturition as a resumption of cyclic increase of muscle tone and other menstrual phenomena controlled by the pituitary gland. This may well be correlated with the atrophy of trophoblast and of decidual cells either or both of which produce a lutein hormone. These observations form parts of one elected interpretation. The ordinary periodic rise of uterine sensitivity is ineffective except when the placental reserve capacity and hormonal production has fallen to a critical point.

We now examine the group of small babies which includes the immature premature individuals. Those one month premature do not vary much from expected ratios because of the predominance of normal fully differentiated small babies. The two-month premature group is not so heavily masked and the negative deviation of placental ratios from expected ratios is

striking. Here then we see some factor at work which produces inhibition of placental growth and leads to premature placental separation through premature uterine activity. Presumably, birth could also result from excess of sensitizing pituitary hormone since this factor has been called upon to explain early abortions.

The large, possibly postmature, babies are interesting in that they all have enormous placentas and despite the size of the baby the ratio is also high. Since drawing this figure more heavy babies have come to hand which would reduce the average placental weights to 836 gm. for the 5,302 gm. class and the corresponding ratios to 0.159. This makes the curve smooth again at the extremities. Here, of course, anoxia cannot be a critical factor since the larger placenta has permitted growth to continue at least until the onset of another potential menstrual period.

In multiple births it is known that crowding in the uterus results in a shortened gestation cycle. The Badgett quadruplets were two months premature. Twins are generally one month premature. We know little about the initiating factors in postmaturity. Some of these very heavy babies represent pathological conditions. Hypothyroidism, diabetes, and erythroblastosis have all contributed individuals to this category.

Genetic factors enter into the maximum potential size, weight, and other growth phenomena as seen both in children and in adults. There is no reason to omit such factors from growth *in utero*. Chick embryos of the same incubation age or state of development, taken from different races, may differ two to four times in volume. However, the attainment of full potential is frequently lacking here as in other genetic operations. Under uniform optimum dietary conditions the size of fetuses vary reciprocally with the number, as shown by Wishart and Hammond (1933) and Reynolds (1939). Here either crowding or inefficiency of the nutrient supply mechanism is responsible. We now report some litters of rats studied for other purposes by Bodansky and Duff (1941) and Sinclair (1942). These are litters produced under a variety of synthetic diets from the list of Cox and Imboden (1936). Calcium and phosphorus are varied. One series is from normal and

the other from parathyroid-ectomized mothers. In general only severely abnormal diets tend to reduce litter size. See Figure 2. As long as the mother is normal there is no marked reduction in the size of the newborn nor of their placentas. When, however, the young are born to parathyroid-ectomized rats the

Fig. 2. Rat Pup-placental Weight Ratios as Altered by Diet

Normal or Parathyroidectomized	Cox and Imboden Diet No.	Ca.	P	No.	Av. Wt.	Plac. Wt.	Ratio	Average Litter
N	7	0.49	0.49	44	5.5	.33	.060	11.0
P				55	4.0	.34	.085	9.2
N	7v	0.49	0.49	30	5.4	.34	.063	11.0
P				11	4.6	.36	.079	11.0
N	16	1.225	0.245	12	5.1	.31	.061	7.0
P				15	5.0	.30	.060	7.0
N	19	1.225	1.225	46	5.1	.30	.059	11.0
P				48	3.2	.29	.093	9.6
N	26	0.017	0.245	30	5.3	.33	.060	7.5
P				38	3.3	.31	.094	7.2

7v indicates viosterol added to diet 7.

added insult does not change placental weights (they have no skeleton), but it does reduce average pup weights markedly, thereby increasing the placental-fetal ratios greatly. It suggests that something similar may be present in small human babies born with large placentas. The lack of calcium in maternal serum as in these experiments is of itself one of the conditions of smooth muscle sensitization.

Summary

It is probable that parturition is initiated by a combination of pituitary hormone sensitizing the uterus together with placental restriction, and is no more precisely periodic than the menstrual cycle itself. Prematurity and postmaturity are less closely related to the placental ratios. There is a definite and nearly lineal relation between full term birth weights and placentas.

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ADENOSINE AND ADENOSINE DEAMINASE*

M. L. Schaedel and F. Schlenk

Adenosine is a ribose nucleoside of special importance. It occurs in all types of cells in higher concentration than any other ribose nucleoside and has a variety of biological functions apart from merely being a constituent of ribonucleic acid. Best known of the physiological functions is the role of adenosine in the adenosine phosphoric acids (Lohmann, 1935), adenosine monophosphate, adenosine diphosphate, adenosine triphosphate; the closely related diadenosine pentaphosphate (Ostern, 1934) and diadenosine tetraphosphate (Kiessling, 1938) (see Formulae, Fig. 1). These compounds act as prosthetic groups of enzymes bringing about the transfer of phosphate. The pyrophosphate linkages in adenosine polyphosphates are energy rich phosphate bonds. Their splitting is attended with a considerable loss of energy (10,000 cal. per mole) which can be made available to other reaction systems requiring energy (Lipmann, 1941). The utilization of this reservoir of energy in the process of muscular relaxation and contraction is of particular importance (Szent-Györgyi, 1947).

Adenosine also is an integral part of coenzymes of biological oxidation, viz., the nicotinamide adenine dinucleotides and riboflavin adenine dinucleotide (Schlenk, 1944). It contributes to the specificity of these compounds in biological hydrogen shifts, but apparently it does not provide for these coenzymes a second role as a phosphate distributor (co-phosphorylase). As yet obscure is the significance of adenosine in form of a thiomethyl derivative (adenine thiomethyl ribose) which is found in yeast (Suzuki, *et al.*, 1924). Another related compound is desoxy-adenosine (Levene, 1931) (adenine desoxyriboside), one of the constituent nucleosides of desoxyribonucleic acid. An interconversion of adenosine and desoxyadenosine has been suggested. The concentration of desoxyadenosine in tissues and its significance do not go beyond that of the other desoxyribose nucleosides.

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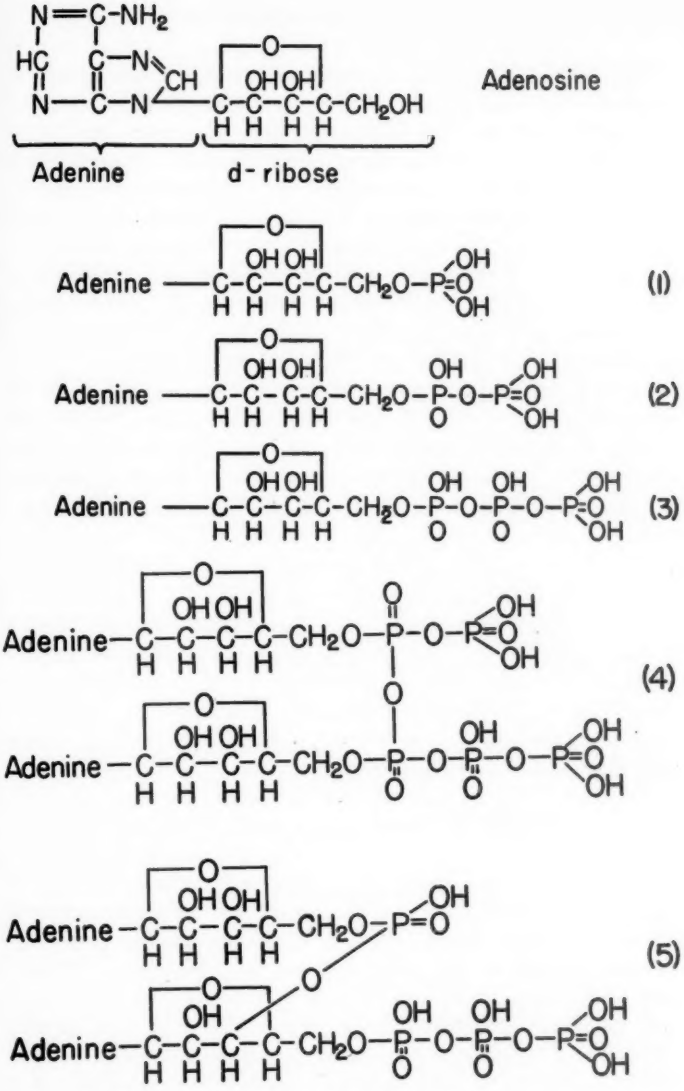


Formulae Fig. I. Adenosine and Its Derivatives:

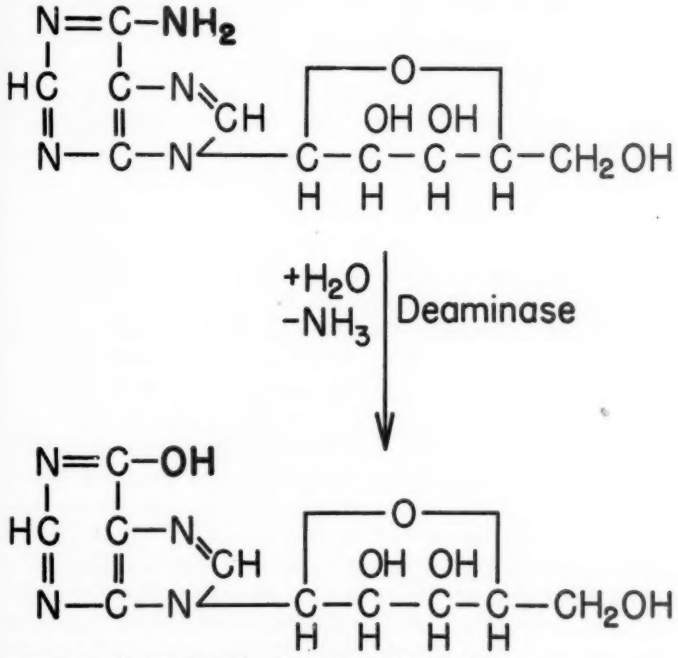
1. Adenosine-5′-phosphate (muscle adenylic acid;
2. Adenosine-diphosphate;
3. Adenosine-triphosphate;
4. Diadenosine-pentaphosphate;
5. Diadenosine-tetraphosphate.

In most organisms adenosine occurs predominantly in combination with phosphoric acid as specified above. In ribose polynucleotides the site of esterification is carbon atom 3 of the carbohydrate. In the coenzymes, however, carbon atom 5 is the place of substitution. The occurrence of an isomeric adenosine-3′-triphosphate in certain autotrophs has been claimed (LePage, 1943). Likewise, it has been stated (Szent-Györgyi, 1947) that the phosphate in coenzymes, besides being linked to the carbohydrate, is attached to the amino group in a labile P-N linkage. These claims have to be taken with caution until a more firm experimental basis justifies them.

From data widely scattered in the literature (Schlenk, 1937) the approximate concentration of adenosine derivatives in the human body can be estimated as 25 to 50 gm. for an average adult. The overwhelming part of it is bound in adenosine triphosphate and in ribonucleic acid; the share of the hydrogen transporting coenzymes is much smaller. Such high concentration is necessitated and explained by the multitude of functions which adenosine derivatives possess.

Purine compounds and nucleosides may be derived largely from nutritional sources. It seems that adenosine also can be synthesized under normal conditions by higher animals, including man, in sufficient amounts, if necessary. Some deficiencies, however, have been reported in the human which seem to respond to the administration of adenosine and its derivatives. One is of nutritional origin and perhaps a sequela of certain states of vitamin B complex deficiency (Spies, 1942). The other seems to involve an impairment of the enzyme system governing the synthesis of adenosine triphosphate and myosine as seen in the disease myasthenia gravis (Szent-Györgyi, 1947). It has been claimed that both disorders respond to the administration of adenosine, but there are two serious drawbacks to its therapeutic use. As yet it has not been possible to devise practical methods of its synthesis on a large scale, and even if satisfactory ways should be found to combine adenine and ribose, the cost of the starting material would be prohibitive. Thus, one will have to depend on adenosine isolated from biological sources. Even more serious is the high pharmacological activity of adenosine

and its derivatives upon intravenous injection (Bennet, 1931; Drury, 1937) which sets a limit to the therapeutic dose. Symptoms observed are cardiac slowing, depression of nodes and conduction of the heart, lowering of blood pressure (largely through direct peripheral vasodilatation), inhibition of intestinal muscle and stimulation of uterine muscle. Since the original observation of Drury and Szent-Györgyi, 1929, considerable research on this subject has been done by numerous investigators. It is remarkable that in the human the safe limit of intravenous administration is about 75 mg. of adenosine or adenosine phosphate (Spies, 1942). This represents an increase of the total adenosine present in the body by less than one per cent. It is obvious then that the pharmacological properties of exogenous adenosine make it impossible to alter significantly by therapeutic administration the total amount



Formulae Fig. II. Deamination of Adenosine to Inosine.

in the body. It becomes clear, also, that nearly all of the adenosine in the body must occur in a special form not exerting these extreme pharmacological properties. Surplus adenosine is either phosphorylated immediately and rendered harmless by combination with protein, or it is converted to inosine by the enzyme adenosine deaminase (Schmidt, 1928) which is widely distributed in the body and is particularly abundant in the small intestine, perhaps to protect the organism from the action of ingested adenosine. Inosine is without pharmacological effect.

It has not yet been possible to reverse the process of adenosine deamination in vitro. Inosine does not yield adenosine when incubated with a large excess of ammonium ion in presence of deaminase. Such a conversion, however, does occur in vivo. It seems that the amino group is derived from a specific donor substance, perhaps asparagine or glutamine.

Attention should be called briefly to the therapeutic use of adenosine and its derivatives in angina pectoris (Herbrand, 1937, 1947) and its possible role in wound healing (Loufbourow, 1942, 1947), tissue injury and shock (Kalckar, 1947).

Experiments on Adenosine Deaminase

In this investigation it was attempted to purify the enzyme adenosine deaminase to obtain information about its composition and properties. Furthermore, a survey was made of its concentration and distribution in the blood of normal persons and patients afflicted with neoplastic diseases, hoping to find significant differences which might be of diagnostic value.

Analytical Methods.—To test the activity of the enzyme 10 mg. (34 gamma mols) of adenosine dissolved in 1.0 ml. of water were incubated with 1.0 ml. of enzyme in 0.06 M phosphate buffer at pH 7.0. The incubation time was one or two hours at 37° C. The amount of ammonia liberated in this system is a measure of enzymatic activity unless the substrate concentration is decreased to very small values (Conway, 1939). For protein determination, Kjeldahl micro digestion and subsequent distillation (Parnas, 1938) or nesslerization was used. The activity of the enzyme is reported here as:

Q_{NH_3} = microliters ammonia formed per hour, per mg. protein.

Purification of the Enzyme.—At the time this investigation was begun, the enzyme adenosine deaminase had been studied in purified form by Brady. Later, Mitchell and McElroy, 1946, and Kalckar, 1947, carried out investigations concurrent with the present work. Besides the isolation steps listed below some experiences with the other methods will be mentioned. In agreement with Brady, 1942, calf intestinal mucosa was found to be the most suitable source for preparation. The content of commercial takadiastase is not always as high as reported by Mitchell, 1946. A sample tested by us did not contain more than traces of the enzyme.

Method A: For the first purification steps the directions of Brady, 1942, were followed. The scrapings of the small intestine of cattle ($Q_{NH_3} = 50$) were dehydrated with acetone yielding a crude material which was stable for many months when stored at low temperature. Further purification with salicylic acid gave a white powder of apparently unlimited stability ($Q_{NH_3} = 300$ to 600). Solutions prepared from it were free from impurities absorbing light in the visible range of the spectrum. The procedure is simple and the product resulting is very useful for further purification.

Dialysis: Dialysis through cellophane membranes is attended with rapid loss of activity. The deterioration is more marked in water solution than in phosphate buffer. It is not possible to reactivate the enzyme by addition of concentrated dialyzate.

Purification with Lead Phosphate.—Adsorption on lead phosphate (Herbrand, 1937, 1947) and subsequent elution with buffer mixtures was found to be a useful purification step ($Q_{NH_3} = 800$ to 1000). Alumina C, modification gamma, proved to be superior and the procedure is described here in detail.

Adsorption on and Elution from Alumina.—Alumina suspension of the modification C-gamma (Willstaetter, 1923) was used. One hundred mg. of deaminase after salicylic acid purification was dissolved in 10 ml. of water. The acidity was

adjusted to pH 5.5 with a few drops of diluted acetic acid under vigorous stirring and cooling. Any resulting precipitate was filtered off and 1.0 ml. of C-gamma suspension was added to the solution. The mixture was then shaken for 20 minutes, centrifuged, and the centrifugate was designated as the first fraction. To the supernatant liquid 2.0 ml. of alumina C-gamma was added with stirring. After 20 minutes of shaking the precipitate (second fraction) was centrifuged and, to the supernatant liquid, 10 ml. of alumina was added as described above. Each fraction of precipitate was washed with a liberal amount of cold water. The washings and the supernatant liquid of fraction 3 contained only a negligible amount of active material. For elution, the precipitates were shaken for 20 minutes with 50 ml. of 0.06 M phosphate buffer of pH 7.5. After centrifugation the supernatant liquid which contained the enzyme, was adjusted with 0.06 M KH_2PO_4 solution to pH 6.5. Most of the active material was found in fraction 3 with some found in fraction 2. The highest purity achieved by this procedure was $Q_{NH_3} = 6000$. The solutions resulting were water clear and suitable for use in spectrophotometric measurements (Kalckar, 1947). They were stable for several weeks when stored at low temperature.

Some other purification steps were attempted. Some of them may prove useful for the isolation of deaminase from other sources. They also give some information about certain properties of adenosine deaminase.

Tannic Acid.—The enzyme can be purified by fractional precipitation with tannic acid solution. This method was applied both to the crude extract and to the salicylic acid purified material. Particular care has to be exercised in the decomposition of the precipitates with acetone to avoid impairment of the enzymatic activity.

Coagulation of Inert Protein by Heat.—Some of the contaminating proteins will coagulate on heating the solution to 55° C. (Brady, 1942), while deaminase remains intact. Often, however, these proteins are removed previously by the salicylic acid treatment.

Removal of Inert Protein by Acidification.—Adenosine deaminase is rather sensitive to acid conditions below pH 4.

With observance of precautions such as speed in operations, cooling, vigorous stirring, the solution may be acidified with 0.1 N acetic acid to pH 4.4. Inert protein was settled out and could be filtered off. The solution was brought back immediately to pH 7 by adding 0.1 N NaOH. An increase in purity of the crude solutions from $Q_{NH_3} = 90$ to $Q_{NH_3} = 210$ was observed with a loss of only 15% of the total enzyme present at the outset. Application of this step to the salicylic acid purified enzyme resulted in little gain of activity.

Fractional Precipitation with Acetone.—The activity of denosine deaminase in purified form is very sensitive to acetone. This effect may account for the losses of active material by acetone treatment after salicylic acid precipitation. In the source material and in crude extracts, however, the enzyme seems to be protected from acetone denaturation by the bulk of other colloids present.

Fractionation with Ammonium Sulfate.—This method applied to a 1% solution after the salicylic acid purification failed to give satisfactory results. Loss of activity was unavoidable, since the precipitates had to be dialyzed to remove adherent ammonium sulfate.

Method B: While we were engaged in preparation of deaminase according to scheme A, Kalckar, 1947, reported an outline for purification of intestinal deaminase which is almost identical with the scheme recommended by Schmidt and Thannhauser, 1943, for the preparation of intestinal phosphatase. The steps in Kalckar's outline were as follows: digestion of intestinal mucosa with trypsin in presence of toluene; repeated precipitation with ammonium sulfate; dialysis in phosphate buffer; fractional adsorption of deaminase on alumina (the phosphatase remains in solution); elution with buffer; dialysis. We were able to confirm the data of Kalckar. The loss of active material is considerable, but the final purity is very high ($Q_{NH_3} = 20{,}000$). The solutions obtained were slightly amber. It is of interest to note that both ammonium sulfate precipitation and dialysis were used in this method while attempts to apply these steps in procedure A resulted in failure. The type of inert material present in the preparations seems to be of decisive importance for the stability.

Composition of Adenosine Deaminase.—To test the preparations for uniform composition, electrophoretic studies with the Tiselius-Klett apparatus were carried out in this institute by Mr. Wirt W. Smith. Even our best preparations were not entirely uniform in the electric field. The analytical results reported below, therefore, were of qualitative nature and hence restricted to a few problems.

Presence of Carbohydrate.—Hydrolysis of deaminase by acid yielded a significant amount of reducing carbohydrate as measured by the Somogyi-Shaffer method (Good, 1933). It seems possible that by interspersing carbohydrate among the amino acids of the protein molecule of deaminase a protection against the proteolytic enzymes of the small intestine is secured.

Examination for Vitamin Content.—Several of the deaminating enzymes contain vitamin derivatives as prosthetic group. An examination of adenosine deaminase seemed indicated therefore. We are most grateful for the coöperation of Dr. B. M. Guirard, Austin, Texas, who has tested two of our preparations ($Q_{NH_3} = 10{,}000$, and $Q_{NH_3} = 21{,}000$) for their vitamin content. The techniques described by R. J. Williams and his coworkers, 1941, were used. No significant amounts of the following vitamins were found: thiamine, riboflavin, folic acid, biotin, inositol, nicotinic acid, and para-amino benzoic acid. The possibility remains, however, that a vitamin prosthetic group is present in adenosine deaminase. It may be different from the vitamins listed, or it may be bound so firmly that it is not rendered available for microbiological assay by preliminary treatment such as digestion with takadiastase and papain (Williams, 1941).

Determination of Adenosine Deaminase in Blood

Our hope to find differences between blood samples from normal persons and tumor cases was based on the consideration that in malignancy excessive amounts of nucleic acid units, among them adenosine, should be found in the blood stream. An increase in adenosine deaminase might then be expected.

After a series of preliminary experiments, the following technique was adopted: To 1.0 ml. of adenosine solution containing 10 mg. (34 gamma mols) of adenosine in 0.06 M phos-

phate buffer of pH 6.5, one ml. of freshly drawn oxalated blood was added. The mixture was incubated at 37° C. for 2 hours. A control experiment (blood + 0.06 M buffer) was carried out in every instance. The set was duplicated if enough blood was available. After 2 hours, the reaction was stopped by addition of 0.1 ml. of 1 N HCl. The amount of ammonia liberated was determined by distillation with an excess of 20% Na_2CO_3 solution in the apparatus of Parnas, 1938, collection in an excess of 0.01 N HCl and subsequent titration of the remaining acid with 0.01 N NaOH. Complete splitting of the adenosine is indicated by liberation of 34 gamma mols of ammonia (equivalent to 3.40 ml. of 0.01 N HCl). The ammonia formation in the control experiments always remained small ($<$ 1 gamma mol) as compared with the experiments (5 to 15 gamma mols of NH_3). The values given in Table I are corrected for the blank ammonia liberation. Freshly drawn blood samples were used exclusively, since storage even in the cold decreases the deaminating power noticeably within a few hours. There was no difference between oxalated and non-oxalated blood. Serum and plasma were found to contain very little of the enzyme. Most of the deaminase is confined to the erythrocytes (Conway, 1939; Drury, 1937) and the data in Table I include, therefore, the erythrocyte counts. The deaminase activity in healthy persons was found rather constant from day to day. It varies very little with the time of the day or with food ingestion.

The results listed in Table I were obtained with patients admitted to the cancer detection clinic of this hospital, the diagnosis being established here. Blood samples of 80 persons (15 of colored race) ranging in age from 20 to 80 years were examined.

The data indicate that the concentration of adenosine deaminase in human blood varies greatly even if the differences in erythrocyte count are considered. There is no significant alteration in various states of malignancy.

Summary

The properties and biological significance of adenosine and its derivatives are discussed. The enzyme adenosine deaminase

TABLE I-A

ADENOSINE DEAMINASE IN BLOOD

Cancer Patients

Male

Age	Race	Site or type of malignancy	R.B.C. $x10^6$	Adenosine split, per cent	
				per ml. of blood	per $1x10^9$ erythro-cytes
68	W	Basal cell ca. (face)	5.22	35.2	6.73
53	W	Brain	2.06	35.0	17.00
74	W	Skin	5.03	47.7	9.48
46	N	Penis	4.80	40.2	8.41
60	W	Mouth	4.72	33.3	6.96
39	W	Bladder	5.23	31.5	6.02
53	W	Lymphosarcoma	5.85	34.3	5.85
52	W	Lymphosarcoma	4.66	34.4	7.37
58	W	Ca. orbit	5.39	58.5	10.80
69	W	Ca. nasophar.	5.06	41.7	8.24
70	W	Bladder	4.20	37.5	8.94
65	W	Tongue	5.47	35.3	6.45
65	W	Lip	4.58	20.3	4.42
46	W	Skin	5.08	27.5	5.40
46	N	Mandible and neck	3.60	29.7	8.28
67	W	Lip	4.53	35.7	7.88
56	W	Prostate	3.87	31.1	8.03
74	W	Rectum	5.35	32.7	6.13
68	W	Throat	4.92	41.6	8.48
50	W	Prepuce	6.12	48.9	7.97
42	W	Lip	4.69	45.5	9.70
38	W	Melanoma	5.67	59.3	10.48
64	N	Prepuce	4.16	39.1	9.38
65	W	Ca. alveolar margin	5.66	23.2	4.11

TABLE I-B

Cancer Patients

Female

Age	Race	Site or type of malignancy	R.B.C. x10⁶	Adenosine split, per cent	
				per ml. of blood	per 1x10⁹ erythro-cytes
27	W	Breast	4.72	15.8	3.35
54	N	Epithelioma (labia)	3.83	19.9	5.18
31	N	Vagina	4.89	38.6	7.89
55	N	Cervix	4.18	31.1	7.43
53	W	Cervix	4.98	20.2	4.05
52	W	Breast	4.19	27.9	6.60
38	W	Nodulotoxic goiter	4.38	43.8	10.00
36	W	Neck	4.94	38.7	7.78
67	N	Cervix	4.79	33.9	7.07
53	W	Fibrosarcoma (orbit)	4.56	39.5	8.64
49	N	Cervix	2.73	22.6	8.27
65	N	Cervix	3.80	19.6	5.16
52	N	Breast	3.68	32.7	8.90
28	W	Cervix	4.16	33.4	8.04
67	W	Ovary	3.31	32.9	9.94
39	N	Rectum	4.22	40.0	9.46
37	N	Cervix	4.19	46.6	11.08
48	W	Cervix	4.84	39.4	8.13
46	W	Breast	4.45	43.8	9.85
35	W	Cervix	3.71	43.8	11.80
49	N	Cervix	2.52	22.4	8.88
45	N	Cervix	4.50	35.3	7.84
64	W	Fundus	4.46	37.0	8.32

TABLE I-C

ADENOSINE DEAMINASE IN BLOOD

Normal Persons

Male

Age	Race	R.B.C. x10^6	Adenosine split, per cent	
			per ml. of blood	per 1x10^9 erythro-cytes
36	W	5.35	36.6	6.85
59	W	5.10	46.4	9.10
45	W	5.21	40.0	7.68
23	W	4.91	36.3	7.40
70	W	4.37	21.9	5.02
80	W	4.95	27.6	5.57
66	W	4.64	42.4	9.13
40	W	4.46	45.1	10.30
57	W	4.72	38.3	8.12
74	W	3.97	31.3	7.90
25	W	5.55	53.2	9.58

TABLE I-D

Normal Persons

Female

Age	Race	R.B.C. x10⁶	Adenosine split, per cent	
			per ml. of blood	per 1x10⁹ erythro-cytes
37	W	4.66	16.7	3.60
20	W	4.46	26.6	5.97
28	W	4.40	33.3	7.58
44	W	5.29	26.1	4.93
55	W	4.59	35.7	7.78
41	W	3.78	27.8	7.36
32	W	4.64	28.8	6.20
74	W	4.82	24.5	5.08
24	W	4.70	38.6	8.22
37	N	4.34	28.1	6.48
32	W	4.86	16.4	3.38
57	W	4.59	33.3	7.26
29	N	4.57	39.6	8.67
43	W	5.50	45.8	8.34
54	W	4.74	42.2	8.90
41	W	4.42	62.8	14.20
51	W	4.47	39.6	8.88
43	W	3.53	23.3	6.60
39	W	5.01	31.6	6.32
22	W	4.73	29.1	6.14
38	W	4.76	46.1	9.70
36	W	4.75	41.6	8.77

Average of adenosine splitting in per cent per 1 x 10^9 erythrocytes:
Normal persons: male, 7.88; female, 7.64.
Cancer patients: male, 7.98; female, 7.90.

has been purified. Some data on its properties and composition are reported. No significant difference in deaminase content was found between normal blood and blood from patients suffering from various types of malignancy.

The authors are grateful to the clinical staff of the M. D. Anderson Hospital for Cancer Research for help in securing blood samples used in this study. Our work has been aided particularly by Dr. C. P. Coogle, Miss A. Fisher, Miss A. Hanselmann, Miss S. Nagai, and Miss C. B. Schwabe.

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THE PROBLEM OF SYPHILIS IN THE NEGRO RACE*

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Syphilis is one of the great diseases of mankind. To our knowledge it spares no race, provided there has been adequate exposure to infection. It is a fair assumption that all peoples react to the infection in much the same way, although the pattern of clinical response may vary significantly among races as it does among individuals. All men may have syphilis of the skin and bones, of the brain and spinal cord and of the heart and great blood vessels. It is only the relative frequency with which the disease localizes in these organs and organ systems which distinguishes one race or one individual from another. The preponderance of localizations in the integumentary or in the nervous system may give to one race, or one individual, a disease of greater severity than it does to another. Lesions of the skin or the bones are usually benign, while those of the brain and spinal cord or of the heart and great blood vessels are serious and often fatal.

For the Negro these differences are not sufficient to render the disease more hazardous to him than to the white man. In some respects the Negro develops the less injurious disease of the two races. But it is the tragic and painful truth that syphilis offers a greater threat to the Negro than to the white people of America.

In considering the racial implications of syphilis it is not the individual Negro or the individual white man who concerns us most. It is the community of Negroes or Caucasians, who comprise a geographical unit, that determines the force of morbidity which syphilis exerts on the race. It is the disease in the mass of population that makes it a major problem of health.

First, let us consider the characteristics of syphilis in the Negro. At what age does he contract the disease? How, and at what rate, does the disease progress from its inception as a chancre to its termination years later in neural and cardiovascular failure? When in the life of the individual are the

*Address to the Postgraduate Assembly of Negro Physicians in Texas, Prairie View, March 3, 1948. From the Department of Dermatology and Syphilology, The University of Texas School of Medicine, Galveston, Texas.

various clinical phenomena to be expected? What is the relative susceptibility of organs to disease and of what significance is the pattern of localization to the individual and thus to the race? These are important questions. Some of them we shall attempt to answer.

It is commonly assumed, and perhaps with some prejudice, that the Negro contracts syphilis at a much earlier age than does the white man. He does contract the disease earlier in life—about four years earlier—but not at the very early age that one is led to believe from prevalent impressions.

In a study of a clinic population in Baltimore, Maryland, it was found that the mean age and its standard deviation of the Negro male at the time he presented the chancre was 25.1 ± 0.4 years. The age of the youngest patient was 14 years and that of the oldest, 54 years. In the same clinic the mean age and its standard deviation of the white male was 29.5 ± 0.6 years, a difference of 4.4 ± 0.72 years. The ages ranged from 14 to 69 years. Because of their earlier maturity, females are younger when they are infected. The mean age of Negro females was 22.3 ± 1.4 years, and that of white females was 25.6 ± 1.6 years. The ages ranged from 14 to 44 years in Negro females, and from 19 to 59 in white females.

One can only speculate on the reason for the earlier appearance of syphilis in Negro than in white individuals. It is unnecessary to assume that differences in susceptibility exist. There is no evidence indicating any immunologic dissimilarity to explain the ease of infection. It seems reasonable to suppose that exposure at an earlier age accounts for the differences between races, as it does between the sexes.

The relatively late age at which the disease is contracted in both races introduces an interesting problem. Assuming that coital contact has been promiscuous under circumstances which would permit infection, we do not know how long after sexual experience begins that a person may be expected to contact syphilis. It seems likely that the risk of infection is not too great, and that within limits the older the individual the greater is the risk. Regardless of this gap in our knowledge, there is no doubt that sexual promiscuity is the one important cause of infection.

Let us turn to the disease and to the chronology of its progression. The first clinical evidence of infection is the chancre. This lesion marks the portal of entry of the infecting organism. Once it was thought that the disease at this stage was localized. Now it is known that even before the chancre appears the infection has already become generalized. It was demonstrated that the blood is infectious for man before the chancre develops. In one instance, when the information was gained fortuitously through the transfusion of blood, the blood of a donor transmitted syphilis to the recipient 20 days before the appearance of a chancre on the penis of the donor. There is no period, unless it is immediately following exposure, when the infection is localized. Since early dissemination of the infective agent is demonstrable in man, it is reasonable to assume that foci of disease in organs such as the brain and aorta are established very early and soon after infection takes place. Viable *Treponema pallidum* has been isolated from the spinal fluid when the only clinical sign of disease was a chancre. The organisms can also be found by animal inoculation of spinal fluid in secondary syphilis even in the absence of any demonstrable abnormality in the fluid. Were it possible to examine as critically the aorta at this early period in the disease, it is altogether probable that spirochetes could be found in the wall of this blood vessel.

These observations are of significance to a proper understanding of the natural history of syphilis. From them one must conclude that whatever may occur late in the disease, even at a remote period of time—in the brain and spinal cord, or in the heart and blood vessels—is foreshadowed by events in the first days of infection.

The course of syphilitic disease is continuously progressive. There is continuity between the first entrance of the pathogenic organisms and the primary, secondary, latent, and tertiary stages, even though this is not evident in the clinical phenomena of the disease. The only clinically adducible evidence of this continuity is the positive serological test. It is to be remembered, however, that this continuity may be interrupted either spontaneously or artifically by treatment, and the order of progression altered by the absence of one or more

clinical phenomena of infection. The disease may fall below the clinical horizon and there be no way of localizing the foci of infection. This generalization has but one exception. When the nervous system has been invaded the presence of asymptomatic neural disease may be demonstrated in abnormalities of the spinal fluid. In some cases the disease may remain clinically latent throughout life, and in many persons it would seem that the infection spontaneously becomes extinct.

But what of the person whose disease is progressive beyond the early period of activity, when the injury to tissues is minimal and often transient, and after the quiescent months or years of latency? To such persons there comes a time when the signs of disease again appear in what is called the tertiary stage. There may be gummatous lesions of the skin and bones, and of other structures where the injury is of small importance to the health of the patient. Even when allowed to progress without treatment, lesions of these structures usually resolve spontaneously without too much functional disability.

There are, however, the serious localizations. These appear later than do the benign lesions, and in persons who have escaped involvement of the skin and bones. They are the terminal phenomena of infection as they are also the fatal events in the disease. They occur in the central nervous and cardiovascular systems.

Chronologically, degenerative disease of the nervous system emerges earlier than disease of the cardiovascular system except that tabes dorsalis and aortic insufficiency frequently exist concurrently. General paralysis of the insane appears at about the fourteenth year of infection in the Negro male and the eleventh year in the white male. Tabes dorsalis usually appears later in both races; in the Negro at eighteen years and in the white male at fifteen years after the chancre. In males of both races cardiovascular disease emerges at approximately eighteen years after the onset of infection. In the life of the patient this is between the forty-third and forty-eighth year, at a time when the effects of age may also add to the causes of disability. With paralytic dementia, tabes dorsalis and aortic valvular disease constitute the critical episodes of syphilis. It

is with such phenomena that the weight of morbidity should be measured in appraising the importance of syphilis in the two races.

There is an impressive reciprocal relationship between Negro and white males in the frequency of neural and vascular syphilis. White males fall victims to general paralysis more often than do Negro males. Around 19% of all tertiary cases in white males are thus affected. Less than 3% of Negro males have cerebral involvement of this nature. Tabes dorsalis with or without primary optic atrophy accounts for about 19% of all tertiary cases in both races.

Although the Negro is less often afflicted with serious neural disease, he fares less well than the white man in the frequency of vascular disease. The excess of cardiovascular localizations in the Negro male is about 15% over that of the white male. Among tertiary cases in Negroes, approximately one-third are aortic insufficiency or aneurysm. For white males the percentage is sixteen. Considering both neural and vascular lesions, the weight of the infection as potentially fatal disease thus strikes a balance slightly in favor of the Negro.

If, as individuals, Negro males escape with less serious disease than do white males, the situation with respect to the race as a whole is much less favorable. The prevalence of syphilis in the two races differs widely. Not only is there more syphilis generally in the Negroes of the United States, but there are extreme regional variations which in themselves carry special significance. They may indicate the basic causes for the divergency between the races.

It is the amount of syphilis in the Negro population rather than any racial peculiarity of localization which poses a problem of major importance to the health of the race and to the nation as a whole.

It was not until the examination of selective service registrants for the last war that any adequate knowledge was had of the size of the public health problem due to syphilis, and of the localities where that problem is greatest. The facts are such as to impress all of us with the seriousness of the situation and with the necessity of adopting measures for its correction.

On the bais of the first 1,895,778 reports on serological blood tests from the entire United States, it was estimated that of Negro males between the ages of 21 and 35 years 252.3 per 1,000 had syphilis. Among white males of these ages the rate was 17.4 per 1,000. In other words, there was 14.5 times as much syphilis in the Negro as in the white population. These are staggering figures for both races. They might well give us cause for sober reflection. Whether the burden of disease falls upon the Negro or the white race, the total weight, regardless of race, rests upon all the people of this nation. It is a problem for all the people, not just a part of the people.

Since we are in Texas, let us look at the situation in this state. The rates for both races in Texas are the second highest of any state in the Union. Only Florida has a higher Negro rate and New Mexico a higher white rate. Among Negro males between 21 and 35 years of age, the rate in Texas was 343.3 per 1,000, and among white males it was 53.4 per 1,000. That is to say, approximately 35 out of every 100 Negro males and 6 out of every 100 white males had syphilis.

Our unfortunate position is better appreciated if we look at the rates in the states having the best records. In Rhode Island the Negro rate was 91.8 per 1,000 as against 343.3 per 1,000. In Wisconsin where the white race fared best, the rate was 6.4 per 1,000 in contrast to 53.4 per 1,000 in Texas.

It should be possible to estimate from these rates the approximate number of persons who might be expected to develop neural and vascular syphilis within the next 20 years. But this would serve no useful purpose for the present discussion. The general problem is already fully apparent. It is to find and treat as many infected persons as possible, and as soon and as effectively as we can.

It may be that the antibiotic penicillin offers the most suitable agent for this purpose. From the standpoint of the public health and the spread of infection it seems certain that penicillin is the treatment of choice. It can be administered within a few days in amounts sufficient to suppress the infection, if not to destroy it. This can be achieved without injury to the patient. And it seems not unlikely that even though penicillin fails to cure the disease in all persons, the results of its use

will at least approximate those obtained from the conventional metallic drugs. The fact that it is possible to administer the full amount of penicillin within a few days makes it superior to arsenic and bismuth, which require months for their safe administration. The longer the time which is required for treatment, the fewer are the patients who complete it. To reduce the time of treatment with arsenical drugs to a period comparable with penicillin so increases the risk of serious and fatal reactions as to eliminate the arsenical drugs as the choice for the rapid treatment of syphilis.

By treatment the sources of infection can be reduced, but it is utopian to believe that they can be eliminated altogether. Therefore, further protection against the disease must be sought through the use of prophylactic measures by those who would expose themselves to unknown sources of infection.

The fundamental problem in the elimination or control of venereal diseases is the reduction of sexual promiscuity. To reduce sexual promiscuity is to change human behavior. However noble and desirable this task may be, it is one not to be easily or promptly achieved. The defects of sexual behavior are deep-rooted in the elemental urges of man. They are conditioned by ignorance, by bad economic conditions, and by social influences largely beyond our control. Many issue from the primitive imperfections of our cultural life. The advancement of mankind from poverty and ignorance to comfort and wisdom, and to a life of freedom has indeed from the beginning of time been a painfully slow and often disheartening experience.

The control of syphilis cannot await the correction of fundamental human faults. As with other infectious diseases, sanitary measures now at hand, or to be developed, must be employed for the protection of the public health. Mechanical and chemotherapeutic agents must be utilized. The development of such measures has received attention in the past; their investigation continues.

In penicillin there is the promise of an effective preventive agent. It is not too great a hope that this antibiotic in some form to be taken by mouth will come to serve this purpose. If this can be achieved, then in the next great mustering of

men, or, it is hoped, in their peaceful pursuits, protection against syphilis and gonorrhea may be as effective as was the protection against malaria by atabrin during the war just ended.

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THE CONCENTRATION OF HUMAN POLIOMYELITIS VIRUS BY PRECIPITATION WITH METHANOL*

MORRIS POLLARD AND MARY S. FINEGOLD

Many techniques have been applied to the problem of concentrating viruses in order that their chemical constituents might be studied and that antigens and vaccines might be prepared therefrom. The sedimentation and concentration of viruses by differential high speed centrifugation and their study by electrophoretic methods often require elaborate and expensive equipment, in addition to highly trained personnel for servicing this equipment.

The concentration of viruses by chemical procedures has yielded techniques which have, in many instances, contributed to a better understanding of these agents and to their control. Many of these techniques are complicated and require elaborate manipulations to attain the end result. Some involve adsorptive treatment with calcuim phosphate (Salk), activated carbon (Carlson), potassium urate (Parodi), and red blood cells (Hirst). The use of protein precipitating agent such as protamine (Chambers), alum (Bodily), ammonium sulphate (Clark), sodium sulphate (Herrarte), and acetone (Howitt) have yielded satisfactory results; the latter three being used with poliomyelitis virus. The precipitation of murine and human strains of this virus at its isoelectric point (4.6) has also been reported (Thieler, Bourdillon).

Recently a technique for the concentration and purification of influenza virus by precipitation with methanol has been described by Cox, *et al.* According to their findings methanol appears to precipitate the protein of the tissue suspension, including the infective agent, with minimal denaturation of the latter. While this technique is simple, it requires rigid control over the concentration of methanol, the temperature, the pH and the time for maximum precipitation of the virus.

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*Presented at the Semi-annual meeting of the Texas Branch Society of American Bacteriologists, November 1, 1947. Supported by a grant from the National Foundation for Infantile Paralysis, Inc. Received for publication March 12, 1948.

We applied this concentration technique to the influenza B virus and the results of Cox, *et al.*, were easily confirmed (Chart I). It it obvious that comparative virus titrations on crude and on concentrated preparations resulted in proportional increment in end point titratability of the latter.

CHART I

CONCENTRATION OF INFLUENZA B VIRUS BY METHANOL, 25%

First Titration—RBC Aggultination

	10	20	40	80	160	320	640	1280	2560	5120	10,240	20,480	40,960	81,920	163,840	327,680	655,360
Crude Virus	—	—	—	—	—	—	—	—									
Conc. 1/10 Vol.	—	—	—	—	—	—	—	—	—	—	—	—					
Supernate	Negative																
Crude Virus	—	—	—	—	—	—	—	—	—	—	—						
Conc. 1/10 Vol.	—	—	—	—	—	—	—	—	—	—	—	—	—				
Conc. 1/100 Vol.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Supernate	Negative																
Crude Virus	—	—	—	—	—	—	—	—									
Conc. 1/10 Vol.	—	—	—	—	—	—	—	—	—	—	—						
Repcpt. 1/10 Vol.	—	—	—	—	—	—	—	—	—	—	—						
Supernate	Negative																

TECHNIQUE

The technique of Cox, *et al.*, was applied by us to human poliomyelitis virus. Ten per cent saline suspensions of mouse brain stems and spinal cords infected with the M.E.F. strain[1] of human poliomyelitis virus were centrifuged at 18,000 rpm for 20 minutes, and the supernatant fluid was then transferred to a water bath at 0–5°C. Cold methanol (C.P.) was stirred gradually into this supernatant fluid, and this mixture was left in the ice bath for three hours. A flocculation appeared shortly after the addition of the methanol. After three hours this "floc" was sedimented in a refrigerated angle centrifuge

[1]Kindly supplied to us by Dr. P. K. Olitsky, Rockefeller Institute for Medical Research.

at 4,600 rpm/30 minutes. The supernate was carefully removed for testing and was then discarded, the sediment was resuspended in an equal or reduced volume of phosphate buffer solution (0.2 M.) and the virus was eluted from the resuspended "floc" during one hour at room temperature. The suspension was then centrifuged at 2,000 rpm/15 minutes and the supernate was removed to test for virus content.

The test for poliomyelitis virus was made by inoculation of 10 Gram Webster strain white mice (0.03 cc intracerebrally) which were observed daily during the following thirty days for evidence of infection. Fifty per cent end points were calculated according to the technique described by Reed and Muench.

Results

Preliminary experiments with M.E.F. human poliomyelitis virus have indicated that this virus can survive treatment with methanol (Chart II). From these results, it appears that final

Chart II

SURVIVAL OF M.E.F. HUMAN POLIOMYELITIS VIRUS AFTER TREATMENT WITH METHANOL

% *Methanol*	*Infective Titer by Mouse Inoculation*			*Supernate*
	10^{-2}	10^{-3}	10^{-4}	
Untreated Virus	4/4*	4/4	4/4	
15%	4/4	4/4	4/4	4/4
20%	4/4	4/4	4/4	0/3
25%	4/4	4/4	4/4	1/4
30%	4/4	4/4	4/4	0/4

*Numerator indicates number infected; denominator, the total number inoculated.

volume methanol concentrations of less than 20% did not extract the virus from the supernatant fluid, since the supernatant fluid retained a considerable amount of infective material.

A study of the time necessary for the maximum precipitation of virus indicated that as little as forty-five minutes

flocculation time was necessary for recovery of most of the virus in the final eluate (Chart III). Shorter periods have not yet been studied.

Studies of the pH at which will occur the maximum elution of virus from the "floc" have indicated that within the range of pH 6 to 8 there was little quantitative difference in the amount of virus recovered in the buffer solution (Chart IV). At pH 4 it appears that a considerable amount of virus has either been retained by the "floc" or has been inactivated.

A 10% brain and cord suspension of infected mouse cords was concentrated to 1/100th of its original volume. The titratable 50% end point was thereupon increased from 10^{-5} up to and past 10^{-9} (Chart V). A similar cord suspension was reflocculated three times with methanol (25% volume) and while the virus survived this treatment, some of it remained in the supernatant fluid (Chart VI).

CHART III

FLOCCULATION TIME—25% METHANOL—CONCENTRATED TO 1/2 VOLUME

Time	*Mouse Infectivity Titer*			
	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Untreated Control	4/4	4/4	4/4	4/4
45 Minutes	3/3	3/3	3/4	2/3
1.5 Hours	3/3	3/3	3/3	2/3
3 Hours	3/3	3/3	3/3	2/3

CHART IV

EFFECT OF pH ON ELUTION OF VIRUS FROM PRECIPITATE—25% METHANOL

pH Eluate	*Mouse Infectivity Titer*				*50% End-point*	*Supernate*
	10^{-3}	10^{-4}	10^{-5}	10^{-6}		
4	1/4	1/4	0/4	0/4	Less than 10^{-5}	0/4
6	2/4	3/4	1/4	0/4	$10^{-4.3}$	1/4
8	2/2	2/3	1/4	2/4	$10^{-4.75}$	0/4
Control	2/4	1/4	1/4	2/4	$10^{-3.77}$	

CHART V

CONCENTRATION OF POLIOMYELITIS VIRUS—
25% METHANOL/3 HOURS

Mouse Infectivity Titer

	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	50% End-point
Crude	5/5	5/5	5/5	2/5				10^{-5}
1/100 Volume	.....	4/4	5/5	5/6	5/5	5/5	5/5	10^{-9}
Supernate	.....	2/5						

CHART VI

REPRECIPITATION AND CONCENTRATION OF POLIOMYELITIS VIRUS—25% METHANOL/3 HRS.

Precipitated	*Concentration*	*Mouse Infectivity Titer*							
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
1	1/10	4/4	4/4	4/4	4/4				
Supernate	2/4								
2	1/100	4/4	4/4	4/4	4/4	4/4	4/4	1/3	1/4
Supernate	3/3								
3	1/100	4/4	4/4	3/3	3/3	4/4	3/4		
Supernate	4/4								

DISCUSSION AND SUMMARY

The technique described by Cox, *et al.*, is a simple procedure, one for which most laboratories are well equipped. When a suspension of M.E.F. strain of human poliomyelitis virus was reduced to 1/100th of its original volume, there was an inordinate increase in concentration of virus as determined by its titratable end point. Whether this is due to an enhancement of potency by exclusion of inhibiting substances, or to a dissolution of virus aggregates is a point which has not yet been clarified.

In the experiments reported herein, more virus was eluted from the "floc" at pH 6 to 8 than at pH 4. This is in accord with the findings of Wenner who reported that poliomyelitis virus could be resuspended easier in a medium of pH 8 than pH 4. Perhaps the virus fails to enter the solution due to the proximity to its isoelectric point (4.6). It seems less probable

that the virus has been inactivated since Loring and Schwerdt found that poliomyelitis virus was stable in solutions of acid at pH 1.6.

It appears, from the results attained, that 25% methanol, at 0–5°C., for three hours, and elution of virus at pH 7 offer satisfactory conditions for survival and concentration of M.E.F. strain of human poliomyelitis virus.

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HORMONAL RELATIONS TO NEOPLASTIC GROWTH WITH REPORT OF A CASE STUDY*

J. B. Ivers, C. M. Pomerat, and H. W. Neidhardt

I. Review of the Literature†

The structural relationships of useful naturally occurring steroid derivatives to carcinogenic compounds has provided one of the most tenable theories for the origin of certain neoplastic processes (Selye, 1943). With the hope that at least part of the mystery of the etiology of malignancy could be solved with a clear understanding of the physiology and chemistry of hormones, many investigators attacked the problem from that approach.

Out of thousands of articles it has now become rather clear that at least three members of the endocrine system play a definite role in relation to neoplasms. The anterior pituitary in its regulation of other endocrine organs is necessarily of profound importance. Ball and Samuels (1938) showed that the absence of pituitary control caused a slowing down of cancer growth but did not attempt to explain whether such a result was due to an actual absence of pituitary hormone or due to a lack of control over the other endocrine glands.

So far as is known now, the thyroid, parathyroids, and thymus glands do not play any special role in the etiology of cancer. However, it should be kept in mind that a definite relationship exists between the thymus and gonads, the exact nature of which is not precisely understood.

The greatest interest and emphasis has been placed on the gonads and their various hormones. In this field the most

*From the issue Culture Laboratory, Department of Anatomy, and Department of Pathology, The University of Texas Medical Branch, Galveston, Texas. Aided by a grant from The American Cancer Society. Received for publication March 18, 1948.

†Submitted by J. B. I. as a senior surgical paper in April, 1947. Since the preparation of this review, the invaluable symposium on "Endocrinology of Neoplastic Diseases," edited by Twombly and Pack, has come to our notice. This report should prove a fresh stimulus for further intensive investigations on the relation of hormones to malignancy associated with the reproductive tract.

encouraging findings, in terms of therapeutic possibilities, have resulted from studies of cancer of the prostate gland. When it was shown that androgenic hormones secreted by the interstitial cells of the testes cause prostatic development, the natural conclusion was that elimination of androgens might stop the growth of prostatic neoplasms. This was accomplished by castration, either by surgery or by irradiation. The patients experienced almost immediate subjective relief and marked amelioration of symptoms. However, the cancerous growth was only temporarily retarded and eventually caused a fatal outcome. As an adjunct to castration therapy, the administration of estrogens was tried with essentially the same results. The rationale of the use of estrogens is that they supposedly neutralize androgens, cause testicular atrophy, and suppress androgens elaborated by the adrenals. Therefore, the current trend in the treatment of cancer of the prostate is the relief of urinary symptoms, castration, and estrogen administration.

Endocrine therapy has also become important in cancer of the breast. Many workers have attempted to show an etiological relationship between carcinoma of the breast and estrogens. In experimental animals this is a definite etiological factor that cannot be neglected. On the other hand, research workers have repeatedly warned against transferring the results of animal experimentation too freely to the field of human malignancy. Bischoff, Long, and Rupp (1942) showed that theelin caused a slight increase in the number of spontaneous mammary cancers in the Marsh-Buffalo strain of mice. These investigators point out that the Marsh-Buffalo strain seems to be more resistant to theelin than other inbred strains.

Geschickter (1939) reported the production of mammary cancers in a strain of mice which did not develop spontaneous mammary cancers. Boger (1946) concludes that estrogens are the hormones which exercise the predominant influence over the mammary gland and, therefore, postulates that estrogen is the dominant factor in the etiology of breast cancer. Therefore, the removal or neutralization of this exciting agent should be the rationale for hormone therapy of breast cancer. Cramer and Horning (1939) reported that ovariectomy decreased the

incidence of spontaneous mammary cancer in mice but did not totally prevent it. They reported further that adrenalectomy produced changes in the mammae which were oppositive to the effect of estrogen. They therefore postulate that the adrenals and ovaries act synergistically and in the absence of one the effect of the other will be diminished. Without going into more detail, endocrine therapy, primarily in the form of castration, has come to play a part in the management of breast cancer as well as prostatic adenomata.

Adair, *et al.* (1945), in a review of a large number of cases of breast cancer in women who were castrated, reported improvement in approximately 15 per cent with retardation of tumor growth for about two years. The most striking results were obtained in young women. The results in breast cancer in males have been spectacular. The relief of symptoms and temporary regression are comparable with the results seen in castration therapy of prostatic carcinoma. Loeser (1941), approaching the problem from a slightly different angle, reported that testosterone propionate decreased the incidence of spontaneous breast cancer in Strong A mice. This strain of mice has a spontaneous tumor incidence of about 83 per cent. He also reported four cases of testosterone therapy in women. Three of the women had undergone radical mastectomy with later local recurrences. These lesions were either removed surgically or by irradiation. Following removal of the tumor growths, testosterone was given and no recurrences occurred in four years. In one case, with widespread metastases, only symptomatic relief was obtained with testosterone therapy. In two cases of hopeless carcinoma of the breast treated with a total dosage of 2400 mg. of progesterone, no symptomatic relief was experienced and no change was noted in the gross appearance of the tumors.

Heiman (1945) has reported more encouraging results. Using the R III strain of mice, which in his hands was found to have a spontaneous breast cancer incidence of 54 per cent, progesterone injected subcutaneously in mice 2 to 6 months old in a total dosage of 6.0 mg. reduced the tumor incidence to 16.6 per cent. Also, worthy of note was the fact that the

tumors that did develop did so much later than was found in the control group. When progesterone was combined with testosterone propionate, 2.0 mg. of each, the tumor incidence was further decreased to 6.25 per cent. Spayed animals with progesterone and testosterone propionate injections failed to develop any tumors. He goes so far as to suggest the administration of small doses of progesterone and testasterone prophylactically to women with a family history of breast cancer.

Adair (1947) reported on the use of testosterone propionate on 200 cases of cancer of the breast. He found that there was no beneficial effect when small doses were used. However, in a series of 48 patients treated with a daily dosage of testosterone propionate there was striking improvement, both symptomatically and by radiological evidence. The final dosage chosen was 100 mg. of testosterone propionate three times weekly. Moreover, it was found that if the malignancy had spread to soft tissues such as lung, liver, brain, and skin nodules, little beneficial effect could be expected. When the metastases had invaded primarily the skeletal system, marked improvement could be anticipated in most cases. The repair of bony destruction can be followed by X-ray to the point where the bone appears normal. In addition, the patients felt much better and pain was decreased or abolished. During treatment, it was found that the blood calcium level at times increased to as high as 17 mg. per cent and the alkaline phosphatase rose to 15 mg. per cent. He concludes that testosterone seems to be longer lasting and more efficacious than X-ray therapy, but does not claim that testosterone propionate is a permanent cure.

The foregoing is intended as a brief review of what has been done with hormones with respect to malignancy of the prostate and mammary glands. Keeping in mind the results obtained and conclusions reached, it is the object of this paper to project those findings to the uterus and ovaries. Literature upon the subject of ovarian and uterine neoplasms, in contrast to those of the breast and prostate, is rather scarce and inconclusive. However, some interesting and significant facts have been brought to our attention by animal experimentalists and clinical investigators.

Taylor (1944) has written a most excellent review of the relationships between sex hormones and the etiology of tumors of the uterus. He points out that clinically it is very difficult to establish evidence that abnormal ovarian function is associated with tumor growth but some facts must not be overlooked. He lists the following points: (1) A clinical history is very important, espeeially with respect to previous pregnancies, normal or abnormal menstruation, or menopausal disturbances. (2) Histological and pathological studies of reproductive organs associated with pelvic diseases may reveal endocrine dysfunction. For instance, the high output of estrogen of a granulosa-cell tumor associated with endometrial hyperplasia is significant. The endometrium is a delicate indicator of the status of sex endocrine function. (3) Clinical therapy has also yielded some evidence to support a hormonal abnormality in that bilateral oophorectomy often causes benign tumors of the reproductive tract to shrink, and in some cases even to disappear. This does not prove that the ovaries or their products caused the tumors, but it does show that the ovaries or their secretion products were necessary for continual susceptibility of the organs involved in tumor formation.

Taylor has considered reproductive tract abnormalities that can be induced in experimental animals by hormonal agents in relation to morphologically similar conditions arising spontaneously in women.

Endometrial hyperplasia has long been accepted as a clinical entity and is usually attributed to abnormal ovarian function. This is supported very well by experimental findings. Typical endometrial hyperplasia can be produced in animals by merely injecting estrogenic compounds. More important, however, is the fact that long continued constant estrogenic stimulation is the essential factor rather than a simple excess of estrogens. Also, the absence of progesterone secretion favors the development of endometrial hyperplasia. Endometrial hyperplasia is difficult to produce in animals if progesterone is given simultaneously with estrogens. It is now generally accepted that endometrial hyperplasia in women is due to the same cause as in experimental animals. Schroeder (1915) ascribed this disease

to the long continued action of unruptured Graafian follicles. This has been confirmed. The almost constant finding of endometrial hyperplasia in patients with granulosa-cell or theca-cell tumors adds additional weight to Schroeder's hypothesis. Even more convincing are the clinical therapeutic results. Castration cures endometrial hyperplasia. Conversely, progesterone in large doses also produces a cure. Large doses of an estrogen will produce the condition in women. Therefore, Taylor concludes that if another pelvic tumor is found with endometrial hyperplasia, the conclusion that both are etiologically related cannot be entirely overlooked.

Squamous metaplasia of the endometrium can be produced in experimental animals by estrogen injections. Quite frequently islands of squamous epithelium occur in the endometrium of women who also have endometrial hyperplasia. This finding suggests that squamous metaplasia is a result of abnormal estrogen secretion.

Spontaneous carcinoma of the endometrium occurs very rarely in animals and is very difficult to induce. However, spontaneous carcinomas have been found to arise in uteri of rabbits. These tumors have at best only a remote histological similarity to carcinoma of the uterus in women. Evidence that such tumors are associated with abnormal estrogen secretion is more convincing in women. It is true that very few cases of hyperplasia have been shown definitely to develop carcinoma later, but it is true that a large per cent of carcinoma cases also show endometrial hyperplasia. Even more convincing is the finding that an increasing number of cases of granulosa-cell and theca-cell tumors of the ovary also have carcinoma of the endometrium. Since primary carcinoma of the uterus is relatively rare and granulosa-cell and theca-cell tumors are uncommon, the simultaneous occurrence of both in the same patient cannot be considered mere coincidence.

It should be kept in mind that the ovaries secrete both estrogens and androgens as well as progesterone, and the relative amounts are important. The testes secrete androgens and estrogens. The adrenals also elaborate both estrogenic and androgenic substances. Abel (1945), in a review of the literature,

points out that sex hormones have four basic actions: (1) Sex hormones in the proper amount stimulate homologous accessory organs. (2) Pituitary secretion products stimulate the gonads to function both in hormone secretion and germ cell production. (3) Gonad hormones may have no direct action upon the gonads of either the same or the opposite sex. Their action is thought to work indirectly through the pituitary. (4) Gonad hormones in excess will suppress the pituitary gland which in turn diminishes the sex stimulating factor elaborated by the pituitary. It is well known that excessive amounts of either estrin or testosterone will cause atrophy of the ovaries. The same is true of the testes.

Mazer and Israel (1939) attempted to determine the effect of progesterone upon the ovaries. Using a dosage of 0.5 mg. three times a week, no inhibition of normal pollicular activity was found. However, when 4.0 mg. were given daily for twelve days, or 1.0 mg. daily for thirty days, definite suppression of follicular activity resulted. The relationship between progesterone and follicular activity has already been discussed in the review of Heiman's work.

Lipschutz and his co-workers have done a great deal to bring about a better understanding of the activity of progesterone. He was able to show that continued injections of estradiol into guinea pigs over a long period of time caused the development of fibromyomata, not only in the myometrium but also in other peritoneal organs such as liver, spleen, and intestinal wall. He then demonstrated that progesterone produces antifibromatogenic effects. When progesterone was given simultaneously with estradiol the incidence of fibromyomata was greatly decreased and approached the number occurring in controls. From this he concluded that the normal rhythmic elaboration of progesterone provides a system of "body autodefense" against the tumorogenic action of estrogens. He was further able to show that progesterone possessed a curative effect upon the estradiol induced tumors. In animals that had had 80 days of estradiol treatment with development of fibromyomata, subcutaneous progesterone pellets stopped the growth of these tumors, even though the injections of estradiol were

continued. Also, after 52 days of progesterone treatment the tumors had decreased as much as 40 per cent in size. Furthermore, treated tumors on microscopic examination revealed a loss of peripheral spindle cells and showed widespread hyalinization with only scattered nuclei. Lipschutz also showed that testosterone had essentially the same effect as progesterone, but to a lesser degree.

Goodman (1946), approaching the problem from the clinical viewpoint, has corroborated the work of Lipschutz. He treated seven women having large fibromyomata with progesterone and in all cases was able to show a decrease in size by at least 33 per cent. However, he states cautiously that the decrease in size, although probably due to a decrease in size of the tumor, may represent shrinking of the uterus itself caused by dehydration. The progesterone therapy also controlled the bleeding and normal menses occurred during treatment. An interesting sideline to this subject was suggested by Dosne (1944) who demonstrated that progesterone is inactivated by the liver although the ability of the liver to perform this function is quantitatively limited to biological amounts. This suggests the possibility of abnormal hormonal balance in people whose liver is not functioning properly. Another important approach was suggested by Bischoff and Rupp (1946) who were able to show that cholesterone, an intermediary product in the degredation of cholesterol to progesterone, was carcinogenic whereas progesterone was not.

Jones and Brewer (1941) have challenged the views held by Taylor and his associates during the last decade. They do not agree that there is an etiological relationship between hyperestrinism and carcinoma of the uterus. In a review of 68 patients they reported results opposite to those of Taylor. However, they admit that uterine carcinoma occurs in about 25 per cent of cases of uterine fibromyomata. In view of the fact that Lipschutz and his co-workers have given experimental proof that hyperestrinism causes fibromyomata, the logical conclusion is that hyperestrinism and carcinoma of the uterus are in some way related.

Nelson (1939) has reported experimental results which are contradictory to those of Jones and Brewer. In a series of 88 guinea pigs treated with estrogens, a large percentage developed marked cystic glandular hyperplasia of the cornua and fundi. Adenomatous hyperplasia of the endometrium with the typical infiltration of adenomyosis was also found. The epithelial cells in the glands became metaplastic, penetrated the submucosa and particularly in the cervical region, displayed epithelial pearl formation. In further support, McEwen has shown that estrone in a dosage of 0.5 to 1.25 mg. in corn oil placed directly into the uterine cavity produced squamous metaplasia of the endometrium in about 50% of the rats. Also, subcutaneous injection of 30 gamma of estrone daily over a period of 132 days produced the same results. As a means of control, 1:2:5:6 dibenzanthracene and tar did not produce metaplasia in the male, even when injected directly into the seminal vesicle.

Having reviewed the literature concerning the relation of progesterone and estrone to uterine tumors in which the concensus of opinion indicates that progesterone is antagonistic to the tumorogenic action of estrone, we may consider the effect of androgens since it is well recognized that testosterone is also an antagonist to estrone. Salmon (1938) attempted to establish the effect of testosterone upon the genital tract of the female. Using immature female rats and a single dose of 0.5 to 20 mg. testosterone propionate, the following results was observed: (1) The vagina opened by 72 hours after injection of the hormone. (2) A single estrus quickly followed the injection. There was then a lapse during which no estrus occurred. Estrus again occurred about the same time as it had in the controls. (3) The ovaries showed follicular proliferation within twenty-four hours and corpors lutea were present within ninety-six hours as shown by sections made every twenty-four hours. (4) The preputial glands became enlarged. Nathanson and Rawson (1939) confirmed the findings of Salmon, and also demonstrated that essentially the same effect occurred in adrenalectomized animals and in adrenalectomized-ovariectomized animals. The antitumorogenic action of testosterone as shown by the fundamental work of Lipschutz has already been mentioned as an antagonist to estrone.

Unfortunately, relatively little conclusive clinical research work has been done recently on the antitumorogenic action of testosterone but some studies have been reported. Abel (1945) reports five cases of malignancy of the female genital tract, two being of the uterine body and the other three of the cervix. All five had received previous irradiation or radium therapy and all had recurred. These women were treated with testosterone propionate at a dosage of 150 mg. weekly for varying periods of time. In no case did the tumors regress. However, all of the patients experienced a spectacular subjective change. There was improvement of the mental attitude from depression to cheerfulness, and in some cases, almost euphoria. Pain was decreased or stopped. Some patients gained weight for about six months and then regressed to their initial weight. One patient, who had been admitted for terminal care before testosterone therapy, went back to work after six months of treatment and was still working nine months after she had been admitted to await death. Beecham (1943) reported immediate subjective relief followed sooner or later by the usual outcome. Hirst (1943) added further weight by treating two cases of advanced endometriosis in humans with testosterone propionate. Both had palpable masses, one in the cul-de-sac and one in the left lower quadrant. Both cases responded favorable to treatment with amelioration of symptoms and decrease in size of the tumorous growths. Hirst did not treat any case with progesterone but planned to report this at a later date.

Although the volume of literature dealing with the effects of estrogens upon other organs is very great, observations dealing with the effect of estrogens upon the ovary itself are not numerous. It has been shown many times that excessive amounts of estrogens will cause atrophy and fibrosis of the ovary. However, this is not thought to be due to a direct action upon it but instead to a suppression of the anterior pituitary with consequent diminution in the amount of follicular stimulating hormone. The action of testosterone upon the ovary is believed to be due to the same mechanism, although there may be actual interhormonal chemical neutralization.

In addition to the types of malignancy that can occur in any tissue, special varieties of tumors occur in the ovary. Three of these are hormone producing tumors. The granulosa-cell and theca-cell tumors produce estrogens and are manifested clinically by feminization. Arrhenoblastomata are androgen producing tumors and produce defeminization followed by masculanization. The relationship of estrogen producing tumors to others of the reproductive tract has already been mentioned.

The etiology of granulosa-cell and theca-cell tumors is as obscure as the etiology of any malignancy. The commonly accepted theory is that they arise from a primitive mesenchymal cell nest. No basis for a hormonal etiology has been postulated. The only finding of significance was reported by Lipschutz. He found that when all but a small piece of ovarian tissue was removed from a guinea pig the piece would hypertrophy and eventually result in hyperestrinism with subsequent endometrial hyperplasia. However, these small pieces did not resemble neoplasms.

The accepted treatment of any malignancy of the ovaries is surgical removal, and the best results are obtained by this procedure. Indeed, there is no justification for depriving the patient of surgery in favor of other forms of therapy unless the tumor has spread and a hopeless situation exists. In such cases radio-therapy is the treatment of choice. Therefore, at present, hormonal therapy can be considered only in relation to both surgery and radio-therapy. The rationale for hormonal therapy of ovarian tumors is not as clear cut as in previously mentioned conditions. It may be likened to the rationale of estrone therapy in prostatic carcinoma, that is, to use a hormonal antagonist to the organ involved. Working upon the assumption that testosterone propionate antagonizes the ovary, Wyatt (1945) treated two cases of hopeless ovarian malignancy with that hormone. One was an adenocarcinoma and the patient was given a total dosage of 7,200 mg. of testosterone propionate. The patient experienced almost immediate subjective improvement comparable to estrogen therapy of prostatic carcinoma. However, there was no regression of the tumor and the patient died. The second case received 3,150 mg.

testosterone propionate and essentially the same results were obtained. The author recommended further investigation in this field.

Other tumors of the ovary such as adenocarcinomata, serous cystic papillary adenomata, pseudomucinous cystic papillary adenomata, and the associated Meig's syndrome occur more frequently and lend themselves more readily to experimental investigation. Is there any relation between these tumors and the hormones of the ovary? As yet, there seems to be none, except that these tumors occur in the ovary.

II. Observations on a Pseudomucinous Cystadenoma[1]

A. *Clinical history and pathological findings.*[2]

An approach to the experimental study of abdominal carcinomatosis was suggested by encouraging results obtained in tissue cultures of cells from ascitic fluid (Ivers and Pomerat, 1947). It has been found possible to describe the natural history of cellular transformations of malignant and nonmalignant peritoneal cells under standard conditions of cultivation *in vitro*. Moreover, the addition of various drugs, hormones, and vitamins affords an opportunity for observing the potencies of such cells. Systematic information of this character is being accumulated in our laboratory. During the past year, several specimens of ascitic fluid from a patient with the diagnosis of pseudomucinous cystadenoma have been studied.

Mrs. B. (Hospital #72182) is a forty-eight year old housewife who first came to the John Sealy Hospital on August 8, 1941, with the chief complaint of gradual swelling of the abdomen since November, 1940. During the last three months prior to admission, the abdominal swelling seemed to have become more pronounced and at a greatly accelerated rate. Since November, 1940, the patient had also noticed menstrual irregularity accompanied by an almost constant whitish vaginal discharge. Upon admission the patient had a markedly protruberant abdomen that contained fluid. In addition she

[1]The authors are indebted to Dr. Willard R. Cooke and members of the Department of Gynecology and Obstetrics for assistance in obtaining ascitic fluid.

[2]Material assembled by J. B. I. and pathological data discussed by H. W. N.

had moderate abdominal tenderness but no abdominal pain. On August 19, 1941, an exploratory laporatomy was done and three gallons of reddish-yellow fluid was removed which contained a considerable amount of gelatinous and mucoid material. A bilateral salpingo-oophorectomy was performed. The pathological diagnosis at that time was pseudomucinous cystadenoma of the ovary. The patient had an uneventful recovery and was discharged on September 7, 1941.

On March 14, 1942, the patient was readmitted with a right direct inguinal hernia that was not reducible. The hernial sac was found to contain 350–400 ml. of bloody ascitic fluid and many gelatinous masses attached to the peritoneum. Many of these were removed and sent to the pathologist. The diagnosis was pseudomyxoma peritonei.

The patient was again seen on May 12, 1942, complaining of abdominal swelling and discomfort. Laparotomy was done and in addition to ascitic fluid, it was found that the omentum was adherent to the anterior abdominal wall and the omentum contained many small gray-black opaque nodular masses. The pathological diagnosis was again pseudomucinous cystadenoma of the omentum, transplant.

The patient was seen again in our clinic on August 9, 1944, three years since the first admission. She had a markedly protruberant abdomen accompanied by abdominal pain and tenderness and respiratory difficulty. Laparotomy was again done and eight liters of light straw-colored fluid were removed. The entire abdominal cavity was found to be studded with small pearly nodules showing coalescence and many adhesions. In the pelvis a soft gelatinous substance was present which was removed along with the greater part of the omentum and subsequently examined microscopically. The diagnosis was again pseudomyxomatous peritonei.

Sixteen months later the patient was readmitted to the John Sealy Hospital with a complaint of abdominal swelling, abdominal pain and tenderness. When the abdomen was opened, 2,000 ml. of yellow gelatinous material was removed. The uterus was found to be studded with large and small nodules

and pseudomusinous material. Extensive implants were present on both the visceral and parietal peritoneum and one section of the ileum was partially obstructed. The diagnosis was the same as before. Eight months later her abdominal cavity was drained again, this time yielding 3,500 ml. of fluid. On May 21, 1947, paracentesis yielded 5,000 ml. of brown translucent fluid. Paracentesis was again necessary in November and December of 1947 and in March, 1948.

Significant pathology reports included the following:

8/19/41 (following operation when 3 gallons of fluid was removed from abdominal cavity):

The specimen consists of about 3 gallons of reddish-yellow gelatinous and mucus material in which there is the remains of a large cystic wall which is extremely friable. This mucinous substance appears to have been contained in a cyst, the wall of which appears to be smooth. An occasional area has a firmer consistency, but on section appears to be fibrous tissue with small spaces filled with a similar pseudomucinous material. No definite papillary appearance of these small areas can be made out. One portion of the wall has a very hemorrhagic appearance.

Section shows numerous cystic spaces filled with a pale pink fibrillar material. Some of the spaces are lined by tall columnar epithelium having basal nuclei and cytoplasm distended with secretion. Delicate spaces, and a few collections of lymphocytes and plasma cells, are present. In other places the cysts are lined by a single layer of flattened or low cuboidal epithelium. There are areas in which the cyst walls remain only as faint outlines, no cellular detail being visible.

Diagnosis: Pseudomucinous cystadenoma of the ovary.

8/19/44:

Microscopic examination of section shows omentum in which there are numerous cystic areas of varying size. The cysts are lined with tall columnar cells one layer thick. The nuclei are situated at the base of the cells, and the cells have large clear vacuolated spaces. In the connective tissue interstices, there is a chronic inflammatory reaction with fibrosis and focal collections of lymphocytes.

Diagnosis: Pseudomyxomatous peritonei.

A description of the most recent collection is typical of the chemical findings of her ascitic fluid. The following table was prepared to compare values for Mrs. B. with those of a patient with a diagnosis of anaplastic carcinoma of the ovary.

ANALYSIS OF ASCITIC FLUIDS*

	Pseudomucinous cystadenoma (Mrs. B.) (ovarian)	*Anaplastic carcinoma (Mrs. C.) (ovarian)*
Sp. Gr.	1.022	1.018
pH	7.82	7.52
Inorg. P (mg. %)	0.85	0.716
Total P (mg. %)	0.77	0.785
Free tyrosine and tryptophane (mg. %)	2.55	2.84
Protein N (mg. %)	154.0	120.0
Non-Protein N (mg. %)	7.0	6.0
Cholesterol (Buckhard-Liebermann)	negative	negative
Gmelin (bile pigments)	negative	positive
Glucose (mg. %)	77.0	64.0

The two specimens of ascitic fluid show a remarkable similarity of composition. It is notable that the prosphorus is entirely in the inorganic form.

*Determinations made by Dr. W. W. Nowinski.

The foregoing brief summary of this patient's long history is given as an illustration of a case of pseudomyxoma peritonei of long standing with a definite confirmed diagnosis.

Relatively few articles dealing with pseudomyxoma peritonei have appeared within the past decade. In this condition, the peritoneum displays diffusely distributed mucinous masses which may be either solid or cystic. The condition is usually secondary to pseudomucinous cystadenomata of the ovary, and sometimes to mucoceles of the appendix. The process is apparently partly metastatic and partly reactive in nature. When pseudomucinous material finds its way into the peritoneal

cavity, two processes begin: (1) The reaction of the peritoneum to a foreign substance, and (2) proliferative activity of the newly located cells. Either one or the other of these processes may predominate in producing the clinical picture.

The earliest reporters of the condition recognized only the reactive process of the disease, and the proliferative or neoplastic aspect was not considered to exist. At present, it is often difficult to determine which process is predominating, for in some cases there are undoubted mucinous cellular tumors scattered about the peritoneal surface, while in others, cells are extremely difficult to find. In the latter instances, the question naturally arises as to whether or not the peritoneum itself is capable of elaborating the mucinous secretion. In few of the reports of pseudomyxoma peritonei following mucocele of the appendix has proliferating epithelium been demonstrated in the peritoneum, and cyst formation is usually absent in these cases also. Certainly, the source of the abundant mucoid material is not explained by the small number of cells present.

Ordinary mucins are precipitated by acetic acid while pseudomucins are distinguished by not being so precipitated. Pseudomucins are alkaline in reaction while mucin is acidic, hence pseudo-mucin stains with the acid dyes, *i.e.*, eosin, phloxine, etc. True mucin, on the other hand, stains with basic dyes. Pseudo-mucin preparations have been reported to have the following elementary composition: C, 49.75; H, 6.98; N, 10.20; S, 1.25; O, 31.74%. Like mucin it yields a carbohydrate group on hydrolysis. Various types of pseudo-mucins have been described and are distinguished by differences in solubility. According to Wells (1925), true mucin never occurs in ovarian cysts.

Perforation of the cyst is apparently not essential to the development of pseudomyxoma peritonei and cases have been reported of the condition developing as late as 17 years after removal of the original ovarian cyst.

The patient under consideration in the present report is fairly typical clinically and pathologically in that the disease developed in connection with pseudomucinous cystadenoma and that the implants often were cystic and showed relatively few but very actively secreting tall columnar cells. The case

is also typical in being refractory to treatment although a few subjects have responded to removal of the primary offending lesion and/or by repeated surgical intervention.

The question naturally arises as to whether or not the pseudomucinous cystadenomata associated with pseudomyxoma peritonei are in any way different in their fundamental nature from pseudomucinous cystadenomata not associated with this condition. It certainly would be difficult to rule out the possibility of obscure biological properties or potencies of certain of these rather common tumors which would lead to the development of the baffling picture of pseudomyxoma peritonei. On the other hand, no morphological differences have ever been pointed out between these two closely related entities. In keeping with the importance of constitutional factors involved in cancer, perhaps the individual biochemical constitution may predispose to the development of the picture. Certainly the case herein considered showed no features as far as the primary ovarian tumor is concerned which could be considered morphologically atypical. It is hoped that more light may be thrown upon the problem through utilization of tissue culture and other biological methods now available to the investigator.

B. *Cultivation of cells from ascitic fluid in vitro.*[3]

Tissue cultures of cells from ascitic fluid are made routinely in hanging drop preparations for initial and daily staining in order to observe cellular transformations under simple standard conditions. One drop of an equal amount of the cell suspension and of 7-day chick embryo juice is dropped through a 27-gauge needle onto a sterile cover glass. A similar drop of plasma from young cockerels is added and thoroughly mixed. Helly-Zenker's solution for one hour, or overnight in 10% saline-formal, have been found suitable fixatives. Staining with hematoxylin-eosin-azure is valuable at the outset for the study of eosinophiles but hematoxylin alone is satisfactory for routine observations after the third day of incubation.

The results obtained with 3 specimens of ascitic fluid from Mrs. B. over a period of 14 months in 1947–48 were remark-

[3]Experimental work carried out by C. M. P. and J. B. I.

ably similar in their behavior *in vitro*. In addition to the presence of a few polymorphonuclear neutrophiles, lymphocytes, and monocytes, initial preparations revealed cells with deeply basophilic cytoplasm (Fig. 1), often containing 2 or 3 nuclei (Fig. 2). Clusters of such nuclei without clear cell boundaries (Fig. 3) were common. Evidences of phagocytic activity were occasionally seen (Fig. 4). Secretory material in cells, occasionally binucleate (Fig. 5), were often present. Dense cellular clusters of the types shown in figures 13 and 14 were characteristic. Occasionally a cavity in globes of cells could be demonstrated (Fig. 12). Hanging drop preparations appeared relatively unfavorable for outgrowths from cell clusters, but individual or small groups of cells produced conspicuously long spindle processes beginning as early as 12 hours after incubation. By the 5th day such spindles were often seen to be binucleate (Fig. 6). Graded series showing progressive multinucleation could readily be found (Figs. 7, 8, and 9). The nuclei of such cells were characterized by the presence of dense chromatin and large nucleoli. In contrast to these, the nuclei of some uni- or binucleate spindle cells were larger and contained much less chromatin (Fig. 10). It is believed that these may have originated from mesothelial cells while spindle cells with dense nuclei may have been derived, at least in part, from exudative polyblasts.

In contrast to the cellular aggregations showing pycnosis and little cellular activity in hanging drop preparations (Fig. 11), luxuriant spindle cell outgrowth was characteristic from such bodies in Carrel flasks. For these cultures a clot formed of 0.2 ml. of media was prepared with materials used for hanging drop preparations and a fluid phase was added containing 6 parts homologous supernatant ascitic fluid, 3 parts Tyrode's solution, and 1 part 7-day chick embryo extract. One thousand units of penicillin was added to each flask. Figures 12 to 17 illustrate the behavior of cells in flasks during a short period of observation. No epithelial sheets developed in these cultures. Experience with these flask cultures suggested that they might afford a suitable test object for a preliminary experimental

study of sex hormone therapy under highly controlled conditions in malignancy. Approximately 10 mg. of pure crystalline androsterone, progesterone, and estrone[4] were autoclaved at 10 pounds pressure for 20 minutes in small test tubes. When the glass was cool, 0.5 ml. of embryo juice was added to each tube and mixed vigorously with the hormone for 10 minutes. After wetting the floor of the Carrel flasks with 0.1 ml. of plasma, 0.1 ml. of cells suspended in ascitic fluid and 0.1 ml. of the embryo juice containing the various sterols were added and mixed rapidly. A control was prepared by omitting all crystalline hormones. Four flasks were set up for each experimental condition. Typical results photographed after 4 days of incubation are shown in figures 18 to 25. All cultures containing the androsterone and progesterone showed a tendency to rounding of cells and the presence of highly refractile granules in the cytoplasm (see especially figures 19 and 21). Cultures containing estrone regularly resemble untreated controls. Direct contact with undissolved estrone crystals appeared to produce no injurious effects (Figs. 22 and 23). After 6 days of incubation, fields of estrone crystals were covered by uninterrupted sheets of migrating cells. Unfortunately, it was not possible to extend these observations at this time. The work is now being repeated and elaborated.

Obviously these results have little meaning without further studies on the effect of sex hormones at dosages within the range of solubility permitted by the culture medium. The relative sensitivity of tissues of genital and non-genital, normal and pathological, types must be investigated. It is recognized that the data presented merely suggest a point of attack for the further elucidation of the rationale of hormone therapy as applied directly to human tissues. Ascitic fluid provides an easily handled source of test material, usually in large quantity and less likely to be contaminated than excised tissue specimens. Without the necessity of tedious exacting cutting of tissue for explantation, test cells can be distributed simply in fluid suspension to a quantity of vessels limited only by the physical facilities of the experimental laboratory. Genetically

[4]Kindly supplied by Dr. Erwin Schwenk of the Schering Corporation.

homogenous materials can be studied systematically under conditions in which experimental variables are subject to highly controllable conditions.

Summary

The majority of patients with prostatic malignancy experience marked subjective relief when treated by castration and/or estrogen administration. The number of definitely improved patients with breast cancer after castration is about 15 per cent. Castration following radial mastectomy in cases of early carcinoma decreases the number of recurrences. At present, hormone therapy is not widely accepted in malignancy of the uterus and ovary. Progesterone is an antifibromatogenic agent. Testosterone inhibits endometriosis. Hyperplasia of the endometrium, squamous metaplasia, adenomyosis, and endometrial carcinoma are probably etiologically related to hyperestrinism. No causal relationship between hormones and malignancies of the ovary have been proven. A case of pseudomucinous cystadenoma is described together with a discussion of the pathology of pseudomyxoma peritonei. Cells from ascitic fluid form an ideal object for tissue culture. Under controlled conditions of cultivation their potentialities may be studied. The addition of sterols to tissue culture media offers an opportunity to make a critical study of hormone therapy in the management of carcinomata originating from genital tissues.

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Plate I

Characteristic appearance of cells from the ascitic fluid of a patient with a diagnosis of pseudomucinous cystadenoma.

Cells in hanging drop preparations fixed in Helly-Zenker and stained with Hematoxylin-Eosin-Azure.

Figures 1 to 4, initial; figures 5 to 10, after five days of incubation. Magnification approximately 535 X.

Fig. 1. Common uninuclear cell showing deeply basophilic cytoplasm.

Fig. 2. Cell resembling that of Fig. 1 but containing 3 nuclei.

Fig. 3. Common type of cellular aggregates.

Fig. 4. Binucleate cell with an ingested nucleus.

Fig. 5. Binucleate cell containing a large amount of secretion.

Fig. 6. Typical spindle cell with 2 nuclei.

Fig. 7. Quadrinuclear spindle cell.

Figs. 8–9. Aggregation of nuclei, presumably formed by the process which produced the spindle in Fig. 6.

Fig. 10. A characteristic aggregation of spindle elements: uni- and multi-nucleated cells with densely or lightly staining nuclei.

Fig. 11. Globular cluster of cells showing little tendency to migrate in hanging drop preparation.

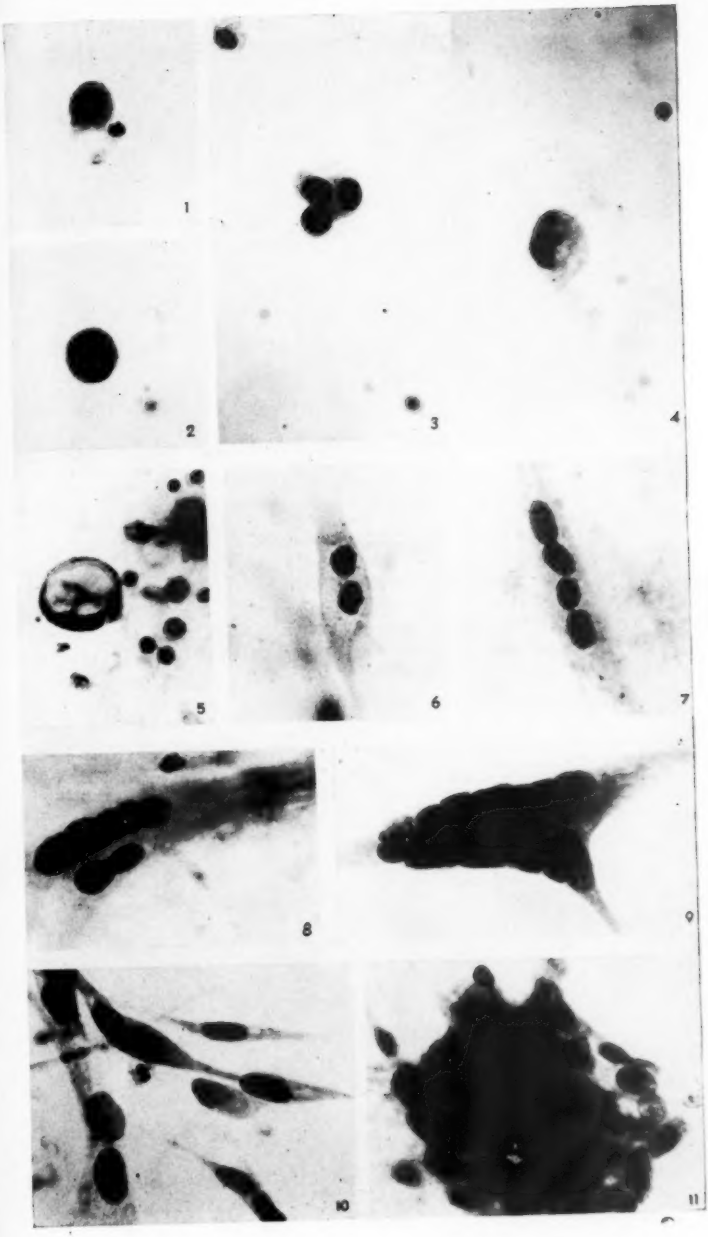
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PLATE II

Living cultures of cells from ascitic fluid (pseudomucinous cystadenoma) in Carrel flasks.

Fig. 12. Photograph taken at the level of the equator of a globe of columnar cells. The blastula-like form of this cystoid body remained unchanged during 2 weeks of observation.

Figs. 13, 14, and 15 show spindle cell migration after 48 hours of incubation from systoid bodies of various degrees of density.

Fig. 16. Typical small aggregation of spindle cells developed on the second day of incubation.

Fig. 17. The same area as in Fig. 16 but on the 7th day. Cells have increased in size and in the number of cytoplasmic granules.

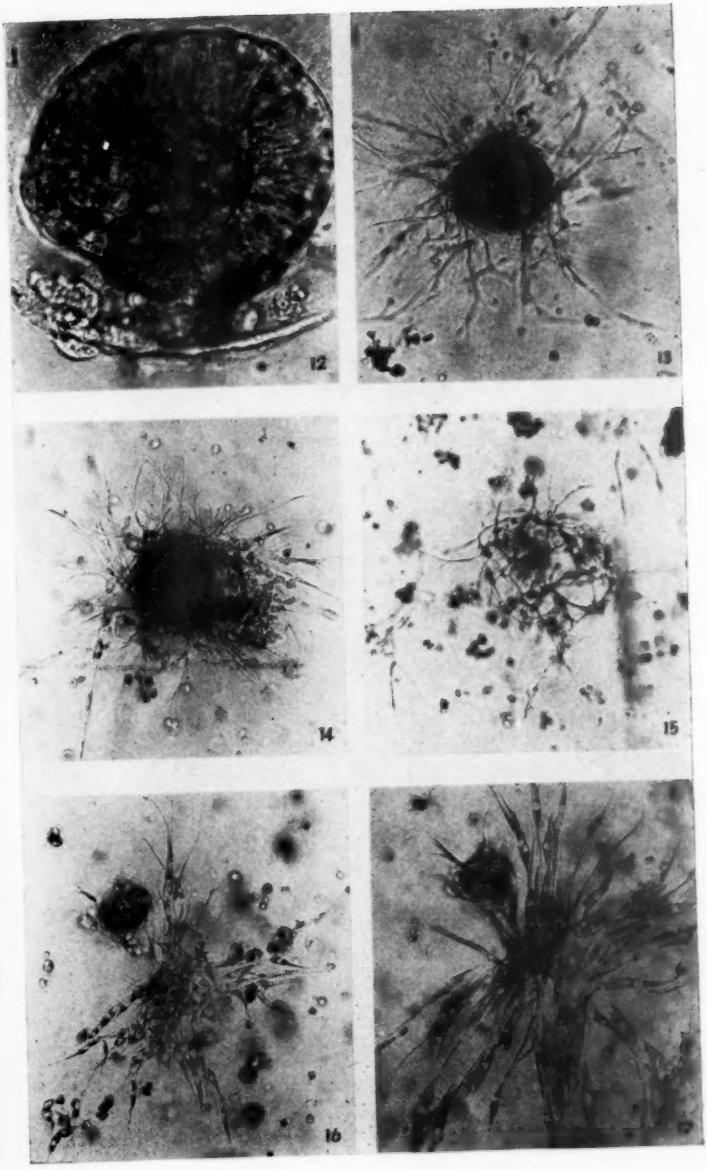


PLATE III

Living cultures of cells from ascitic fluid (pseudomucinous cystadenoma) in media containing various sterol compounds after 4 days of incubation.

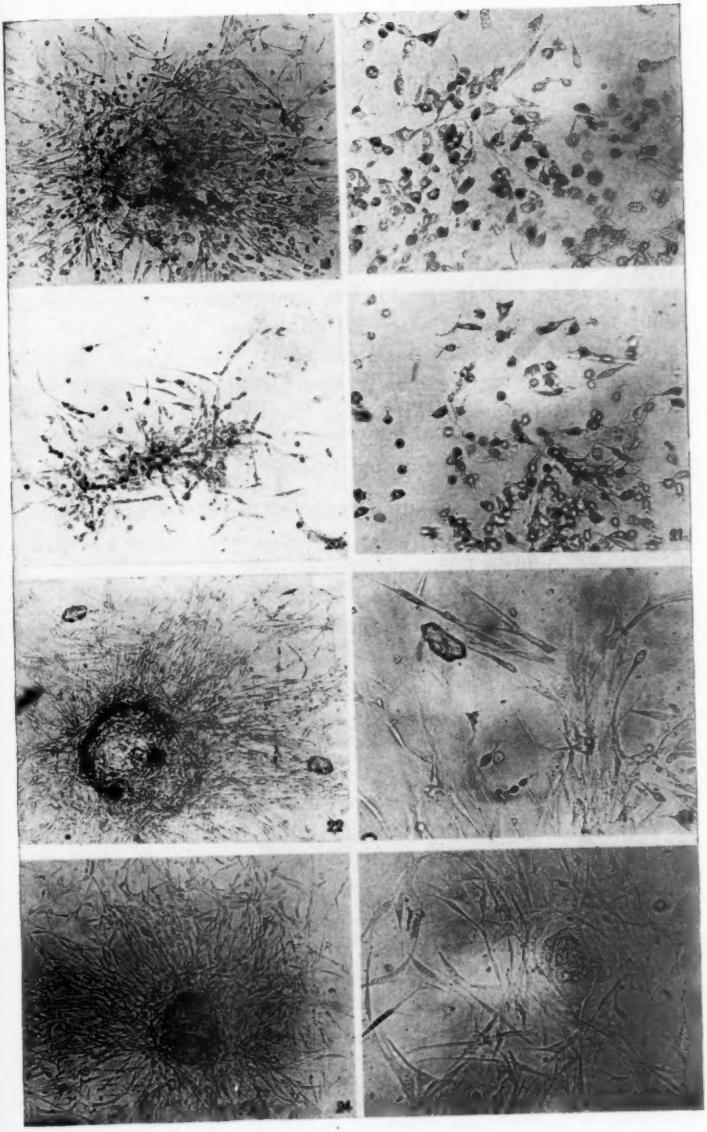
Figures 18, 20, 22, and 24 at approximately 70 X, while figures 19, 21, 23, and 25 at 160 X.

Figs. 18–19. Androsterone crystals added beyond the point of saturation in the culture medium.

Figs. 20–21. Progesterone crystals added beyond saturation.

Figs. 22–23. Estrone. Note presence of undissolved crystals.

Figs. 24–25. Control, free of sterol compounds.



THE TEXAS CITY DISASTER—CARDIOVASCULAR STUDIES, WITH FOLLOW-UP RESULTS*

A. Ruskin and O. W. Beard

Elevated arterial tensions occurred so commonly in the blast victims of the Texas City Disaster of April 16, 1947 that 180 well-documented cases were made the subject of a special report (Ruskin, A., Beard, O. W., and Schaffer, R. L., 1948). Four to seven months after the explosions, 111 patients were re-examined on one or more occasions. The data so obtained together with some previously unpublished cardiac studies form the subject of this paper.

Cardiac Injuries

Because of the tremendous force of the blasts and the fact that many of the examined survivors were within 100 yards of the exploding ships and plants, typical blast injuries of the heart were sought for clinically and on postmortem examination. Such cardiac blast injuries have been reported in air (Cohen and Biskind, 1946), but not in underwater (McMullin *et al.*, 1943), explosions in World War II. They would be expected to manifest themselves in the guise of myocardial or coronary insufficiency, various arrhythmias, tears of any portion of the heart or great vessels, and non-specific electrocardiographic evidences of myocardial damage, such as occur in other non-penetrating injuries of the chest and heart (Bright and Beck, 1935; Wilson, 1943).

If there were any cases of angina pectoris or coronary occlusion and myocardial infarction that could be attributed to the explosions, we were not aware of them. We encountered no clinical or pathological cases of frank myocardial failure. It was impossible to rule out latent myocardial insufficiency in cases of severe toxemia and severe and irreversible shock

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(Wiggers, 1947). Many severely injured and infected patients, whether or not in the shock state, manifested low voltage of the T and, occasionally, of the R waves of the electrocardiogram, especially in the limb leads and in the moribund state (Fig. 1). S-T segment depression and inversion of T_1 and T_4 were also rarely observed (Fig. 2). Electrocardiographic alterations were the exception rather than the rule, as noted in previous series of patients in shock (Burnett *et al.*, 1946). Our electrocardiograms were similar to those previously reported in air blast injury (Burrow and Rhoads, 1944) in showing low voltage of the T and R waves.

Pathologically, besides hemorrhages at the roots of the mesentery, of the lungs, and of the great vessels, Dr. N. D. Schofield (unpublished observations) encountered at least 2

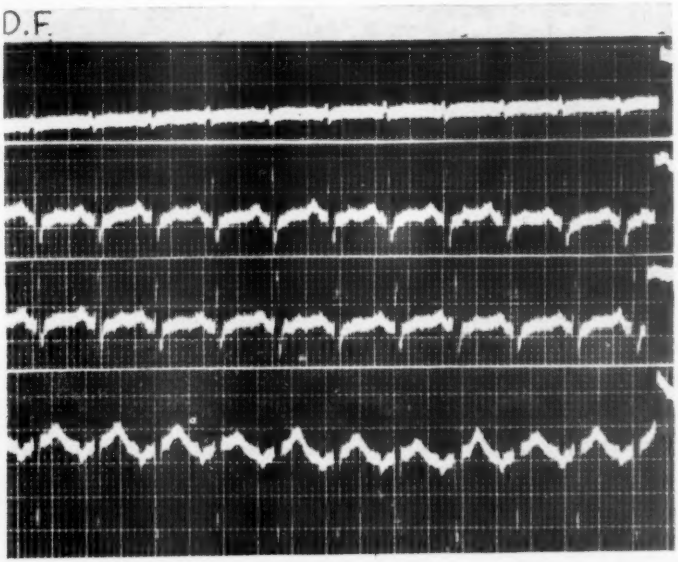

FIG. 1. Electrocardiogram of Case 4, D. L. F., 30, white, male. Leads I, II, III, and IV F taken 3 days after the blasts. Low R_1 and R_4, and low T_1, T_2, and T_3 are evident. Heart clinically normal, moderate hypertension, no post-mortem examination.

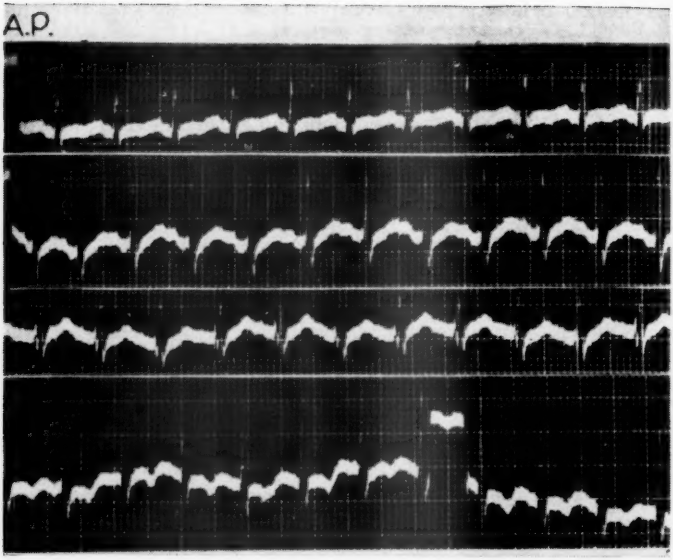

FIG. 2. Electrocardiogram of Case 27, A. P., 18, colored, male. Leads I, II, III, and IV F taken 2 days after the explosions. Depressed S-T segments, and inverted T_1 and T_4 are shown. Heart clinically normal, mild hypertensive state.

cases of possible myocardial "contusion," with myocardial edema and diffuse small ecchymoses and petechiae, involving especially the perivascular subepicardial areas. Both of these patients died within 3 days of the explosions (cases 2 and 3 *infra*). Since both patients were unconscious, we could not be certain that their intrathoracic injuries were due to actual air pressure waves or to unknown missiles. Nor are these cardiac hemorrhagic areas, as the above electrocardiographic changes, clearly due to blast injury rather than to severe shock or toxemia. Yet these are precisely the types of cardiac lesions not infreqently seen in cases of non-penetrating (*e.g.*, steering wheel) injuries of the heart, as well as in previously reported blast injuries (Cohen and Biskind, 1946). We did not see among the hospitalized patients any gross tears or ruptures

of the endocardial, myocardial or pericardial layers of the heart that were not due to penetrating types of trauma. Postmortem examinations of the several hundred victims that died before reaching the hospitals were necessarily incomplete in this regard, so that the percentage incidence of cardiac blast injuries is unobtainable.

Cardiac Arrhythmias

One case of paroxysmal A-V nodal tachycardia occurred in a healthy male 32 years of age who sustained fractures of the femur and 2 ribs in the explosions 5 days previously (case charted in prior paper). The paroxysm ceased immediately after 0.1 mg. of digitoxin was administered intravenously, 35 minutes following a first dose of 0.6 mg. There was no recurrence, and the cardiovascular status was otherwise normal; only the electrocardiogram showed typical digitalis effects upon resumption of sinus rhythm. Among other arrhythmias encountered there were a few cases of infrequent ectopic beats, usually ventricular origin.

Vascular Complications

We saw no cases of frank thrombophlebitis or phlebothrombosis, or of peripheral arterial or venous insufficiency in the blast victims, except for the usual manifestations of shock. At least 2 patients, on the other hand, suffered massive pulmonary embolism (cases 1 and 5 *infra*). In one case (case 1, W.S.R.) the bilateral embolus caused death 30 days after the blasts. In the other (case 5, J.R.D.), the first massive left pulmonary embolus and infarct 14 days after the explosions was associated with the highest rise in blood pressure during the course of his illness (190/130). The second embolus 5 days later, might have caused exitus except for prompt and energetic use of heparin and dicumarol. As it was, bronchopleural fistulae, and hemopneumothorax followed by empyema, developed, and necessitated decortication and lobectomy of the left lower lobe before dismissal.

Postexplosion Hypertension

A striking finding from the cardiovascular standpoint was the occurrence of, usually transient, hypertension of all grades of severity in an actual majority of hospitalized cases. 103 of the 180 closely followed patients presented diastolic maxima of 95 mm. to 160 mm., generally 2 to 28 hours following the blasts (Table I, fig. 3). We could not correlate these rises in diastolic (and frequently systolic) pressures with the degrees of crush or intracranial injury, intravenous blood, plasma, and fluid administration, shock and the post-shock state, and the use of analeptic drugs (epinephrine was not among them). In general the patients who were within several hundred yards of the explosions, and sustained, unprotected by solid objects, the severe force of the blasts, showed the greater height and frequency of diastolic hypertensive peaks (see case reports). A few, of course, died in irreversible shock without any hypertensive phase at all. Ten to thirteen days after the

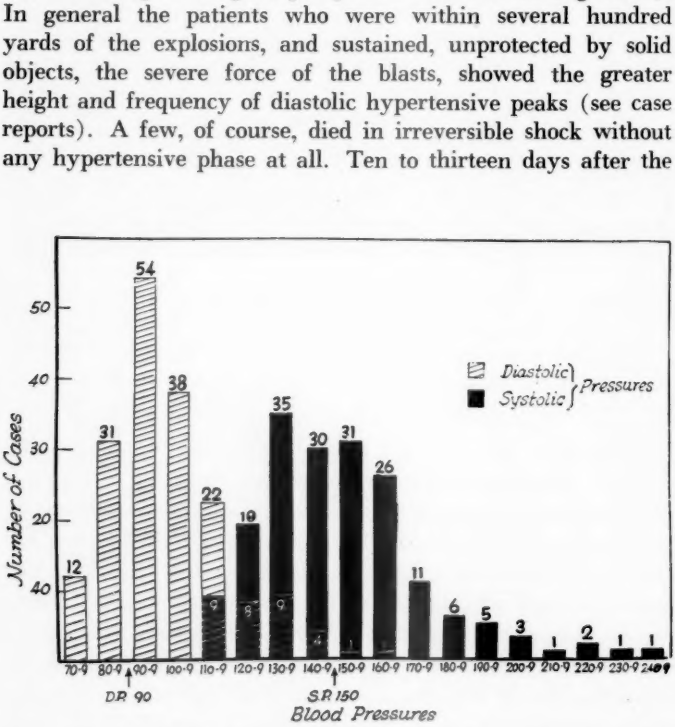

FIG. 3. Distribution of systolic and diastolic blood pressure peaks in 180 hospitalized blast victims the first few days following the Texas City explosions.

TABLE I

Comparison of Immediate Systolic and Diastolic Blood Pressure Maxima of the Blast Victims with the Minima on Follow-up Examinations

	Systolic pressure 150 mm. or more	Systolic pressure 149 mm. or less	Total	Systolic pressure 150 mm. or more	Systolic pressure 149 mm. or less	Total
Diastolic pressure 95 mm. or more	73	30	103	7	17	24
Diastolic pressure 86–94 mm.	12	30	42	2	15	17
Diastolic pressure 85 mm. or less	5	30	35	0	70	70
Total	90	90	180	9	102	111

blasts the blood pressure was normal in 49 of 61 representative cases, or 80%; 5 presented diastolic readings of 95 mm. or over, and the remaining 7 were 86 to 94 mm. Cold pressor tests performed at that time were positive in 39 of 54 cases, or 74% (Table II a).

TABLE II A

Postexplosion Cold Pressor Tests

Postexplosion diastolic maxima	Positive D.P. rise 17 mm. or more	Borderline D.P. rise 14–16 mm.	Negative D.P. rise 13 mm. or less	Total
95 mm. or more	26	7	3	36
86–94 mm.	8	0	3	11
85 mm. or less	5	1	1	7
Total	39	8	7	54

TABLE II B

Follow-up Cold Pressor Tests

Follow-up diastolic minima	Positive D.P. rise 17 mm. or more	Borderline D.P. rise 14–16 mm.	Negative D.P. rise 13 mm. or less	Total
95 mm. or more	16	0	2	18
86–94 mm.	9	0	3	12
85 mm. or less	39	3	12	54
Total	64	3	17	84

Follow-up Data

Follow-up records, up to 8 months after the explosions, were available in 111 patients. The great majority complained of nervousness, various aches and pains, and particularly hypersensitivity to loud noises. Many cases still visited the various out-patient services, orthopedic, neuropsychiatric and rehabilition clinics in particular. The 111 patients were re-examined, often repeatedly, after rest in the sitting position, and following exercise and the cold pressor test, to determine their cardiovascular status. Cardiac neurosis was not infrequent, with palpitation, precordial stabbing and aching pains and sighing dyspnea as pre-eminent symptoms. Organic heart disease, on the other hand, was not clinically evident except for enlarged hearts in 3 cases who had known hypertension previous to the explosions.

Diastolic pressures over 95 mm. after rest (diastolic "minima") were noted in 24 of the 111 cases (22%), as contrasted to the finding of diastolic peaks of this magnitude in 103 (57%) of the 180 originally studied blast victims (Table I,

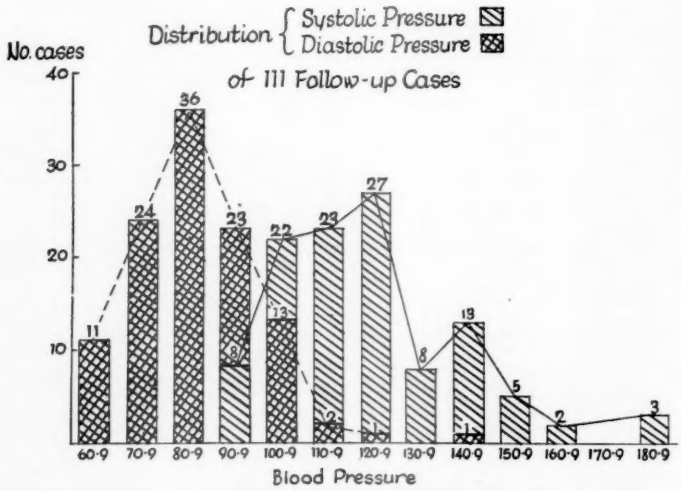

FIG. 4. Distribution of systolic and diastolic blood pressure "minima" in 111 follow-up cases four to seven months after the Texas City explosions.

figs. 3, 4). Under the circumstances of infrequent re-examinations we were not able to obtain significant diastolic peaks to compare with the postexplosion data. This makes doubly significant whatever diastolic hypertensive cases were demonstrated on re-examination, since the readings were the lowest of a series. It was also impossible to compare these diastolic pressure "minima" with postexplosion "minima," since the latter were often very low as a result of shock, hemorrhage, infection, dehydration and other factors. It will be noted, however, that in other respects such as age (Table III), sex and race (Table IV), the groups were quite comparable.

TABLE III

Comparative Age Incidence of 180 Victims of the Texas City Explosion and of 111 Follow-up Cases

Age	1–9	10–19	20–29	30–39	40–49	50–59	60–69	70–79	Unknown	Total
No. — April, 1947	2	20	47	57	20	16	8	6	6	180
No. — Follow-up	0	8	22	37	22	12	8	2	0	111

Age, Sex and Race Factors

The influence of age on the incidence of diastolic hypertension in the follow-up group of cases is of some interest. The great majority of diastolic hypertensives were in age groups of 30 to 59, more especially 30 to 39 (Table V). This is quite comparable to the incidence of postexplosion diastolic peaks, except that transient hypertensive phases were then also noted in a few cases 10 to 29, and even 3 to 9, years of age.

As previously shown for the postexplosion group, diastolic hypertension was more frequently found in the colored patients. 54% of white patients presented diastolic peaks of 95 mm. or over originally and 18% had like diastolic minima in the follow-up series; the corresponding figures in the Negro groups were 69 and 32% (Table IV).

Role of Family and Past History of Hypertension

The influence of positive family history of hypertensive states upon their occurrence in the blast victims is noted in

TABLE IV

Comparative Sex and Race Incidence of Blast Victims with Diastolic Pressures of 95 mm. or Over After the Explosions and on Follow-up Examinations

	Postexplosion diastolic maxima of 95 mm. or over	All cases	Follow-up diastolic minima of 95 mm. or over	All cases
White				
Male	63	112	14	71
Female	15	32	1	12
Negro				
Male	21	30	6	22
Female	4	6	3	6
Total	103	180	24	111

TABLE V

Follow-up Diastolic Pressure Minima Classified by Age Groups

Diastolic blood pressures	10–19	20–29	30–39	40–49	50–59	60–69	70–79	Total
95 mm. or more	0	0	9	9	4	2	0	24
86–94 mm.	0	3	6	0	3	0	2	14
85 mm. or less	8	19	22	13	5	6	0	73
Total	8	22	37	22	12	8	2	111

Table VI a and b. A definite history of hypertension, cerebral hemorrhage, and progressive heart or renal failure in parents, siblings, uncles, aunts, or grandparents was obtained in 29% of 85 blast victims. 92% with this type of positive famliy history presented diastolic peaks of 86 mm. or over soon after the explosions, as opposed to 78.5% of the negative history group; the difference did not appear very significant. In the follow-up group a positive family history was obtained in 28% of 89 cases (Table VI b), a figure coinciding with that of the original blast victims. *All of these, on the other hand, presented rest diastolic pressures of 86 mm. or over, as opposed to only 26% of those with negative family histories.* Hereditary

TABLE VI A

Postexplosion Diastolic Pressure Maxima Classified According to Positive or Negative Family and Past Histories of Hypertension

Diastolic pressures	Family history		Past history	
	positive	negative	positive	negative
95 mm. or over	18	29	3	41
86–94 mm.	5	18	2	19
85 mm. or less	2	13	0	15
Total	25	60	5	75

influence may, it would seem, have a bearing upon the incidence of diastolic hypertensive readings in the follow-up group.

The indicated positive past history of hypertensive states in the blast victims (Table VI a) is possibly much below the actual one. Great efforts were expended in culling doctors' and company records for previous blood pressure readings; the patients and their families were repeatedly questioned. Definite known previous hypertension was found in only 5 of 80 original blast victims. All these presented diastolic pressure phases of 86 mm. or over immediately after the explosions. Sixty of the blast victims with no known previous hypertension reacted similarly. In the follow-up group all 4 known hypertensives presented rest diastolic pressure readings of 95 mm. or over. *Of the 90 follow-up cases with no known previous hypertension 30% presented rest diastolic pressures of 86 mm. or over and 22% 95 mm. or over* (Table VI b).

Blood pressure figures of routine company examinations are available in the case of the Pan-American Refining Co. of Texas City through the courtesy of Dr. T. M. Frank. They represent the population group to which most of the blast victims belong. Diastolic pressures over 90 mm. were noted in 8.3% of 1,723 employees: in 7.4% of 1,558 white, and in 11.5% of 165 colored employees. The stated hypertensive incidence in Negroes is actually too low as some of them were refused jobs because of the finding of high blood pressure. These figures are considerably below those of our follow-up group.

TABLE VI B

Follow-up Diastolic Pressure Minima Classified According to Positive or Negative Family and Past Histories of Hypertension

Diastolic pressures	Family history		Past history	
	positive	negative	positive	negative
95 mm. or more	8	12	4	20
86–94 mm.	2	9	0	7
85 mm. or less	14	55	0	63
Total	24	76	4	90

Dr. Marshall Brucer of the Physiology Department, made statistical comparisons of our group of 71 white males (see Table IV) in the follow-up series with the large white male group of insurance examinees previously analyzed by him (Robinson and Brucer, 1939). Upon adjusting both groups for age distribution there was found to be a 10 mm. of Hg. difference in the mean diastolic pressures between the two groups. The difference of the groups unadjusted for age was 9 mm., indicating a very small variation in the age distribution. The mean diastolic pressure of the 71 males was 83 mm. of Hg., with a standard error of 1.63 mm. The mean diastolic pressure of 7,388 men with a similar age distribution was 74 mm. of Hg., with a standard error of the mean of 0.12. The "t" test indicated a P (probability) of less than 0.00 of this difference being due to chance. A Chi square test was done on the proportion of white males presenting diastolic pressures of 95 mm. or over. In the 7,388 control males there were 3% showing such diastolic pressures. In the 71 males of our follow-up series there were about 20% showing elevations of that magnitude. The Chi square resultant was again much too large to allow this to be a chance difference.

The results of the cold pressor tests are noted last because of their doubtful significance. In our previous report we have pointed out that they bear no significant relation to family, and to some extent, past hypertensive states. We can state the same opinion regarding the follow-up group (Table II b). Moreover, in a group of 50 controls 15 to 60 years of age, 25

hospitalized for other than cardiovascular disease, and 25 ambulatory plant employees who had escaped the explosions, we obtained positive cold pressor tests by our criteria (Table II a and b) in 28 or 56%. This figure corresponds to the highest recorded in the literature, that for aged individuals (Russek and Zohman, 1945). The regular use of a vertical bucket with relatively large amounts of ice cubes may have been responsible, in part, for the marked pressor responses, which were admittedly greater in degree in hypertensive cases and in those with positive family histories of hypertension (see Case Reports). In the light of this high incidence of positive cold pressor tests in the local population, the finding of positive cold pressor tests in 72% of the postexplosion group and 77% of the follow-up group of blast cases (Table II a and b) loses much of its significance. Some of our cases had positive cold pressor tests soon after the explosions and negative tests on later re-examination, as well as vice versa (see Case Reports).

Comment

The outstanding result of the follow-up study is the finding of diastolic hypertension of various degrees in a significant minority of Texas City blast victims several months after the explosions. While previous studies of air blast injuries emphasized the findings of hypotension and bradycardia (Barrow and Rhoads, 1944), those were the immediate effects and not those noted on prolonged observation. It is unfortunate that no reliable figures are obtainable for the incidence of hypertension in the local population or in groups corresponding to the blast victims, except for those quoted above. Only 0.5% of over 60,000 applicants for ordinary life insurance had to be rated up or rejected because of hypertension, according to Dr. Dublin (1935). The incidence of transient diastolic hypertension of over 90 mm. in the Armed Services or the veteran groups has been generally placed below 20% (Levy, *et al.*, 1944 and 1947; Rogers and Palmer, 1944; Silver and Baker, 1946). Our figures are higher. Because of population shifts the group we have been able to follow is constantly getting smaller. This will unfortunately interfere with future follow-up studies,

which would be very important in determining whether the transient hypertension was really provoked on the day of the blast, if its owners represent the potential hypertensive pool in the general population, and, finally, if it will become permanent hypertension in the future.

Known hypertension prior to the explosions was present in only 6.3% (5 cases) of the original group and in 4% (4 cases) of the follow-up cases. It would be hazardous in the extreme to claim that, therefore, 20 or 18% of the follow-up cases developed diastolic hypertension of 95 mm. and over as a result of the Texas City explosion. Our information is not sufficiently exact, nor our re-examinations sufficiently prolonged to substantiate this statement. That some patients *may* have developed hypertension since April, 1947, is indicated from the evidence of previous normal blood pressures on repeated examinations (see Case Reports). Incomplete fundoscopic and renal function studies have been essentially negative in unearthing advancing retinopathy or nephrosclerosis in the blast victims. Electrocardiographic signs of left ventricular strain, which in hypertensive cases, we have found characteristically reversible by tetraethyl ammonium (Ruskin and Lane, 1947), have been noted by us in relatively few cases.

The type of hypertension we are dealing with in either the uneven postexplosion peaks or in the follow-up diastolic valleys is far from clear. In our previous study we have emphasized the possible role of neuropsychogenic, endocrine or chemical, and renal vasoconstrictor factors. The present work has added no new theoretic bases for the past or current findings.

That the uncovered hypertension may be indistinguishable from essential hypertension or may lead to all of its manifestations in the future is indicated by several studies. First, studies of the blood pressure records of the American Armed Services (R. L. Levy, *et al.*, 1946) indicate that transient hypertension, which they found in some 12% of the whole group of over 22,000 cases, leads to permanent hypertension of the essential type 3.6 times as often as in the rest of the group. Secondly, in cases in which the diastolic pressure was found at one time to be above 85 mm., hypertension resulted in 59.7%

at the end of 10 years and in 82.5% in 20 years (Hines, 1940). Thirdly, in re-examining 50 Navy personnel, who had had one reading of 140–156/80–94 1 to 7 years previously, Master (1943) found hypertension in 76%; in 25% the diastolic pressure exceeded 100 mm. Fourth, the weakest, evidence for eventual hypertension, is that immersion in cold water, with the supposed production of renal vasoconstrictor substances, was blamed for the finding of 80% positive cold pressor tests in merchant navy seamen several months to 1 to 2 years after shipwreck by mine or torpedo blasts (Garai, 1945).

On the other hand, only 5 of 33 members of an armored brigade, or 15%, still had a diastolic pressure over 100 mm. 1 to 2 months after a battle in the Western Desert Campaign, whereas 26% of the total group showed this degree of hypertension right after the engagement (Graham, 1945). Moreover, the possibility cannot be excluded that Garai's 15%, Master's 25%, and our 22%, incidence of diastolic hypertension is precisely the incidence of essential and other forms of hypertension in the larger social groups from which the special series were taken. Figures as high as 16.9% of all males, and 23.6% of all females in the general population 40–49 years of age are noted in the literature for the incidence of hypertension of 150/95 mm. or over (Master, 1943). For older age groups they are even higher. That the majority of our hypertensive cases were below the age of 50 (Table V) may or may not answer this criticism. Statistically controlled local studies of various social groups would be of aid in settling this fundamental question.

Case Reports

Note: The following representative case reports may be of value in clarifying certain aspects of the above study. Some hypertensive cases have been charted in the previous report. Typical hypertensive and non-hypertensive cases are included below. The fundi, urine, and blood non-protein nitrogen were essentially negative in all cases; fever up to 103°, and corresponding tachycardia, were usual; anemia (to 50% Hb and 2.5 million RBC) of frequent occurrence. Apathy and semi-stupor, as previously noted in air blast injuries (Barrow and

Rhoads, 1944), were present in the great majority of blast victims on admission to the hospital; actual unconsciousness, on the other hand, was relatively an exceptional occurrence. Severe shock was noted in a minority of cases and promptly treated with intravenous plasma or blood, amounting to as much as 2 liters in the first 24 hours, the average being 1 liter. Morphine was used freely; paraldehyde was substituted in the relatively few cerebral injury cases. Blood pressure spikes were apparently independent of any of the above.

The first explosion of the ship Grandcamp, loaded with ammonium nitrate, occurred at 9:12 A.M., April 16, 1947. This set off the second explosion of the buildings of the nearby Monsanto Chemical Plant at 9:15 A.M. These will be referred to below as the first and second explosion, respectively. In listing blood pressure findings the number of hours (or days) after the explosion that they were taken are indicated by figures in parenthesis.

FATAL CASES (4)

CASE 1. *W. S. R.*, J. S. H. #3590 D, 30, white, male. Was blown out of dragline in the Monsanto plant 200 yards from the ship by the first explosion, then hurtled some 30 yards into the middle of the #1 Compress Building by the second explosion. Never unconscious, he crawled some 500 yards before receiving first aid, despite a compound comminuted fracture of the right tibia and various lacerations. Company examination November, 1946, as several previous examinations: "normal" blood pressure (detailed records destroyed by blast). Family history negative for hypertension.

Blood pressures: *160/140 (3), 160/110 (5), 160/100 (11), *220/130 (16, postoperative), *210/140 (19), *206/140 (21), *180/130 (23), 154/110 (25), 90/64 (47), 128/68 (49), 130/72 (51), 142/96 (75), 149/100, 110/70 (27 days, postoperative).

Cold pressor test (12 days): 118/82 (control), 132/94 (30 sec.), 138/92 (60 sec.), 138/96 (120 sec.), negative or borderline.

Fatal massive bilateral *pulmonary embolism (28 days).*

Post-mortem: J. S. H. #7089: Embolus pulmonary artery and bifurcation, atelectatic and congested wedge in one lung; prevertebral hemorrhage; cerebral edema; passive congestion of viscera. *Heart negative.

CASE 2. *C. H.*, J. S. H. #3535 D, 26, white, male. No history available except of severe blast injuries. Died in 2 days.

Blood pressures: 130/56 (5), 110/80 (6), 104/74 (7), 120/70 (13–14), 120/65 (20), 130/80 (48), died (50).

Post-morten: J. S. H. #7063: Comminuted fractures right femur, tibia and fibula; bilateral pleural effusions; pulmonary hemorrhages, especially perivascular (blast injury?); petechiae kidney pelvis and brain (especially basal ganglia); congestion viscera. *Heart 350 gm., left ventricle 18 mm. thick, *myocardium soft and edematous, *subepicardial small hemorrhages and petechiae, especially perivascular.

Case 3. *D W.*, J. S. H. #3484 D, 49, white, male. No history obtainable except of severe blast injuries. Died in 1 day.

Blood pressures: 74/28 (3), 98/40 (7), 90/50 (8), 60/15 (13), 60/38 (14), 88/40 (16), died (24).

Post-mortem: J. S. H. #7061: Ruptured spleen, comminuted fracture left 9th rib, hemorrhages in lung, especially left lower lobe (as on X-ray) and liver (blast injuries?), 2 linear tears right kidney, heart 320 gm., left ventricle 13 mm. thick, *R. B. C. foci in epicardium and myocardium.

Case 4. *D. L. F.*, J. S. H. #3522 D, 30, white, male. No history obtainable except of severe blast injuries. Unconscious throughout hospital stay. Died 7th day of cerebral concussion, compound fracture humerus, deep lacerations, penetrating wound left eye, pulmonary injury with pneumothorax left. NPN never above 47.5 mg. %.

Blood pressures: 130/80 (6), 140/60 (11), 100/60 (24), 122/56 (26), 130/80 (30), 144/80 (32), 128/70 (41), 130/68 (42), 128/60 (43), *191/112 (86), *160/120 (85), 174/94 (87), 170/110 (88), 156/120 (5days), 132/90 (6 days), died (7 days).

HYPERTENSIVE CASES (23)

Case 5. *J. R. D.*, J. H. S. #3518 D, 22, white, male. Was in Monsanto machine shop 150 yards from ship, hit by flying bricks and concrete, crawled away. Not unconscious, but sustained fractures of pelvis (superior pubic rami), medial malleolus of right tibia, transverse process of 5th lumbar vertebra; multiple lacerations; ruptured left eardrum, draining spinal fluid, Monsanto Co. exams 1947—"normal" blood pressure, as in U.S. Army. Family history negative for hypertension.

Blood pressures: 122/74 (2), 120/88 (6), 120/80 (7), 120/90 (12), 114/78 (35), *132/96 (45).

First massive *pulmonary embolism,* left (14 days): ashen cyanosis, fever, tachycardia (140), dyspnea, tachypnea (42), syncope, hemoptysis, rub, dilated pupils, rolling eyes, EKG changes; *190/130 (¼), 170/90 (2), 168/78 (3), 134/56 (4), 134/58 (5).

Second massive *pulmonary embolism* (19 days): 120/46 (16), 144/28 (24), 112/50 (22 days), 138/78 (28 days), 130/60 (29 days).

Cold pressor test (10 days): 138/78 (control), 142/90 (30 sec.), 144/92 (60 sec.) 150/90 (120 sec.), borderline.

Post-lobectomy (2 mos.): 90/70, 100/72 (½), *112/100 (1), 120/98 (1½), 104/86 (2), 120/92 (2½), 116/88 (3½), 130/88 (4½), 110/80 (5½), 124/80 (27).

Follow-up blood pressures: 105/70 (4 months).

Cold pressor test (4 mos.): 105/70 (control), 130/90 (30 sec.), *130/100 (60 sec.), 110/75 (120 sec.), positive.

CASE 6. *G. L. S.*, J. S. H. #3600 D, 34, white, male. Knocked down in Monsanto Co. office 200 yards from the ship. Not unconscious, but sustained a fracture dislocation of right elbow (olecranon), deep lacerations and multiple puncture wounds of chest, back and scalp. Mother had hypertension. Company exams 1945–6, as numerous U.S. Army exams, revealed "normal" blood pressure.

Blood pressures: 130/80 (9), 140/75 (15), *210/130 (23), 196/88 (26), 200/110 (28), *208/120 (30), 120/80 (33), 126/78 (50), 185/100 (54), 140/90 (120), 126/90 (20 days, postoperative), 126/70 (21 days).

Follow-up blood pressures: *110/80 (4 mos.).

Cold pressor test (4 mos.): 110/80 (control), 120/95 (30 sec.), *120/100 (60 sec.), 120/85 (120 sec.), positive.

CASE 7. *L. E. P.*, J. S. H. #3583 D, 33, white, male. Standing on shore 70 feet from exploding ship, blown and carried by overflowing water and oil for 2 blocks. Not unconscious, hobbled off with fractured carpal bones and talus, left hydropneumothorax and pulmonary atelectasis, ruptured right eardrum. Past and family histories negative for hypertension.

Blood pressures: 130/70 (4), 104/56 (9), 136/85 (10), 110/70 (14), 110/88 (15), 125/70 (16), 100/60, *140/92 (34), 136/80 (35), 142/92 (36), 140/80 (37), 118/75 (72).

Cold pressor test (10 days): 120/80 (control), 120/90 (30 sec.), 120/95 (60 sec.), 130/100 (120 sec.), 140/90 (240 sec.), positive.

Follow-up blood pressures: *110/90–100 (4 mos.).

Cold pressor test (4 mos.): 110/100 (control), 120/100 (30 sec.), *130/120 (60 sec.), 110/100 (120 sec.), positive.

CASE 8. *T. G.*, J. S. H. #4494 D, 50, Mexican, male. Knocked down by a piece of steel 1,000 yards from ship; compression fracture 3rd cervical vertebra. Past (company exams) and family histories negative for hypertension.

Blood pressures: *144/130 (14), 120/100 (5 days), 140/90 (12 days).

Cold pressor test (12 days): 140/95 (control), 170/120 (30 sec.), *190/130 (60 sec.), 190/130 (120 sec.), positive.

Follow-up blood pressures: *120/90 (4 mos.), 120/80 (9 mos.).

Cold pressor test (4 mos.): 120/90 (control), 150/130 (30 sec.), *190/150 (60 sec.), 150/110 (120 sec.), positive.

CASE 9. *N. G.*, J. S. H. #5519 C, 27, Mexican, male. Knocked down by ship explosion while on dock 50 yards away, blown 15 yards into air and bay, hit by debris. Facial lacerations only. Walked 12 blocks to town. Past and family histories negative for hypertension.

Blood pressures: 128/70 (5), 100/68 (11), 124/72 (14), 130/70 (15), *162/110 (16), 138/90 (19), *172/130 (21), 140/90 (23), *162/130 (25), 154/90 (54).

Follow-up blood pressures: *110/70 (4 mos.).

Cold pressor test (4 mos.): 110/70 (control), 110/80 (30 sec.), 110/90 (60 sec.), 120/80 (120 sec.), negative.

Case 10. *E. A. M.*, J. S. H. #3567 D, 39, white, male. On 5th floor in Polystyrene Building of the Monsanto Co. when blown into water and buried by debris of second explosion, whence pulled out by a girl; fractures cervical spine and humeral neck, ruptured ear drum. Past history negative for hypertension (company exams). Older sister—hypertensive; mother had "stroke."

Blood pressures: 142/80 (5), 145/100 (9), 128/90 (110), 120/70 (14), 118/78 (25), 144/99 (29), *160/120, 112/70 (36), 125/85 (78), 128/84 (96).

Cold pressor test (10 days): 112/82 (control), 126/92 (30 sec.), 126/92 (60 sec.), 126/92 (90 sec.), 130/94 (120 sec.), negative.

Follow-up blood pressures: 120/82 (4 mos.).

Cold pressor test (4 mos.): 120/82 (control), 130/100 (30 sec.), 140/100 (60 sec.), 115/82 (120 sec.), positive.

Case 11. *V. E. B.*, J. S. H. #3497 D, 37, white, male. Thrown on ground 30 yards off 25 foot high scaffold. Using a "2 x 6" as a crutch, "surrounded by fire," hobbled over 7-foot cyclone fence to first aid. Unconscious for unknown period; fractured mandible, lacerations, ruptured eardrum. Past history negative for hypertension. Father hypertensive first at age 70, "stroke" at 80.

Blood pressures: 122/86 (2), 118/68 (4), *160/110 (10), 138/68 (14), 134/90 (23), 130/80 (36), 122/88 (48), 122/80 (49), 122/88 (5 days), 140/90 (11 days).

Cold pressor test (12 days): 125/75 (control), 125/78 (30 sec.), *140/100 (60 sec.), 150/84 (120 sec.), positive.

Follow-up blood pressures: 105/85 (4 mos.).

Cold pressor test (4 mos.): 105/85 (control), *105/90 (30 sec.), 110/95 (60 sec.), 105/90 (120 sec.), negative.

Case 12. *W. H. A.*, J. S. H. #3493 D, 36, white, male. Knocked to knees 100 feet from exploding ship, unconscious twice that day. Severe cerebral concussion, fractured ribs, ruptured eardrums, perforating wounds legs. Past history negative for hypertension. (Monsanto Co.) Father and mother hypertensive. Saturated with paraldehyde.

Blood pressures: 170/90 (23), 150/68 (24), 150/96 (28), 144/98 (30), 150/100 (32), 170/90 (34), 152/100 (39), *172/108 (40), 160/100 (41), 160/110 (42), 146/100 (48), *170/110 (52), 170/110 (62), 160/110 (69), 144/70 (83), 122/70 (11 days).

Follow-up blood pressures: *120/85 (4 mos.).

Cold pressor test (4 mos.): 120/85 (control), 125/100 (30 sec.), *140/120 (60 sec.), 120/95 (120 sec.), positive.

Case 13. *S. J.*, J. S. H. #3710 D, 24, colored, female. Several hundred yards from explosions, fracture left foot by piece of steel as ran out of

house. Very nervous since blast. Past history negative for hypertension. Maternal aunt hypertensive.

Blood pressures: *158/98 (7 days), 160/96 (9 days), 132/80 (12 days), 120/70 (26 days), 126/90 (34 days, postoperative).

Follow-up blood pressures: 120/90 (4 mos.).

Cold pressor test (4 mos.): 120/90 (control), 130/115 (30 sec.), *160/130 (60 sec.), 155/115 (120 sec.), positive.

Case 14. *C. W.*, #3619 D, 39, colored, male. Blown into air from dock near exploding ship, and felled by falling brick and debris. Unconscious for short period. Dragged himself away and walked 1 mile to first aid. Compound comminuted fracture left scapula, laceration left lumbar region. Past (Monsanto Co.) and family histories negative for hypertension. Dyspnea, precordial aches (4 mos.).

Blood pressures: 140/80 (9), 112/72 (15), 118/90 (17), 130/98 (29), *144/110 (35), 125/88 (36), 130/88 (38), 130/90 (41), 115/80 (44), 126/94 (87), 120/80 (96), 118/78 (6 days).

Follow-up blood pressures: *150/110, 130/100, 110/80 (4 mos.).

Cold pressor test (4 mos.): 120/85–90 (control), *130/110 (30 sec.), 130/100 (60 sec.), 130/110 (120 sec.), positive.

Case 15. *C. P.*, J. S. H. #5163 D, 50, white, male. Knocked down 2 blocks from exploding ship, unconscious for unknown period; clothes completely blown off, covered by tidal waves of water and floating lumber, walked to first aid. Fractures of skull and 3 ribs, blast injury (?) of lungs (multiple hemorrhages), ruptured eardrums, lacerations. Past (T. C. Railroad Terminal Co.) and family history negative for hypertension.

Blood pressures: 118/80 (10), 110/80 (15), *175/130 (17), 110/80 (30), 150/100 (41), 120/98 (46), 124/82 (49), *140/100 (53), 146/100 (54), 135/95 (59), 140/90 (60), 142/94 (63), 128/90 (64), 132/86 (65), 120/84 (85), 122/96 (8 days).

Cold pressor test (11 days): 100/75 (control), 100/85 (30 sec.), 110/90 (60 sec.), 90/70 (120 sec.), negative or borderline.

Follow-up blood pressures: **145/105 (4 mos.).

Cold pressor test (4 mos.): 145/105 (control), 160/120 (30 sec.), *165/125 (60 sec.), 140/110 (120 sec.), positive.

Case 16. *W. C. T.*, J. S. H. #3609 D, 29, white, male. Knocked down by second explosion on ground floor of Monsanto plant; previously reported case. Severe lacerations arms and legs, severed nerves. Past (Monsanto Co.) and family histories negative for hypertension. Overweight (220 lbs.). Hypersensitive to loud noises (4 mos.).

Blood pressures: 108/72 (2), 98/70 (3), **190/160 (10), 140/110 (11), 90/68 (12), 120/98 (13), 144/108 (14), *164/120 (23), 160/80 (25), 188/90 (28), 136/72 (33), 130/70 (36), 142/98 (47), 110/76 (49), 102/68 (51), 125/90 (10 days).

Cold pressor test (11 days): 142/84 (control), 144/86 (30 sec.), 146/100 (60 sec.), 146/100 (120 sec.), borderline or positive.

Follow-up blood pressures: *120/90 (4 mos.).

Cold pressor test (4 mos.): 120/90 (control), 125/95 (30 sec.), 130/95 (60 sec.), 120/90 (120 sec.), negative.

CASE 17. *P. T.*, J. S. H. #3606 D, 29, Mexican, male. Knocked down 300 yards from exploding ship, not unconscious, carried by tidal wave. Lacerations, ruptured eardrums. Past (Company exams, including 3 weeks before blast), and family histories negative for hypertension.

Blood pressures: 130/90 (2), *135/100 (5), 132/88 (6), 140/90 (9), 128/88 (10), 120/90 (12), 122/80 (24), *150/122 (25), 128/90 (28), 132/96 (30), 120/70 (36), *140/100 (47), 126/78 (49), 120/78 (51), 124/80 (72), 122/70 (96), 110/60 (6 days), 100/70 (12 days).

Cold pressor test (11 days): 112/72 (control), 120/90 (30 sec.), 130/94 (60 sec.), 134/100 (120 sec.), positive.

Follow-up blood pressures: 125/80 (4 mos.).

Cold pressor test (4 mos.): 125/80 (control), 140/100 (30 sec.), *150/100 (60 sec.), 120/85 (120 sec.), positive.

CASE 18. *A. R.*, J. S. H. #3634 D, 35, white, male. Sitting on wharf 30 feet from exploding ship, blown 300 yards into drainage ditch, dazed for some minutes; walked ¾ mile to town; fractured ribs and radius, ruptured eardrum, contusion right kidney. Past history—intermittent slight hypertension (Company exams). Mother hypertensive. Hypersentitive to loud noises, very nervous, headaches (4 mos.).

Blood pressures: 153/75 (9), 148/90 (29), *136/96 (33).

Follow-up blood pressures: *140/100 (4 mos.).

Cold pressor test (4 mos.): 140/100 (control), 170/120 (30 sec.), *200/120 (60 sec.), 170/110 (120 sec.), positive.

CASE 19. *G. Ad.*, J. S. H. #3660 D, 50, white, male. Unconscious for seconds, fractured ribs, lacerations. Past (Monsanto Co., 1945–6) and family history negative for hypertension.

Blood pressures: 164/95 (3), *165/95 (8), 140/78 (10), 140/60 (12), 140/80 (13), 150/62 (16), 142/65 (21).

Follow-up blood pressures: *150/100 (4 mos.).

Cold pressor test (4 mos.): 150/100 (control), 180/140 (30 sec.), **210/155 (60 sec.), 180/115 (120 sec.), positive.

CASE 20. *G. Av.*, J. S. H. #3639 D, 57, Mexican, male. *Firefighter in the hold of the exploding ship*, thrown in the bay and hit by flying lumber; unconscious for 1 hour or so; depressed skull fracture, lacerations, ruptured eardrums. Past history negative for hypertension (*1946 J. S. H. admission; fundi normal). Family history negative. Received 750 cc. plasma first 12 hours.

Blood pressures: 140/96 (8), *158/100 (16), 146/90 (18), 143/92 (20), 130/90 (22, 24), 158/78 (33), 150/90 (34), 140/95 (36), 154/98 (38), 136/94 (39), 150/100 (40), 148/108 (41), 148/110 (45), 160/104 (51), 120/70 (54), 156/106 (56), *166/116 (60), 138/96 (96), 140/110 (7 days), 135/98 (8 days), 150/110 (10 days).

Follow-up blood pressures: **180/145 (4 mos.).

Cold pressor test (4 mos.): 180/145 (control), 210/170 (30 sec.), *200/175 (60 sec.), 175/130 (120 sec.), positive.

Case 21. *G. W. W.*, J. S. H. #3616 D, 52, white, male. Not unconscious, lacerations of eye (eviscerated), thigh and forehead. Past history—"low" blood pressure (120/?) (T. C. Railroad Terminal Co.). Family history—brother died of heart disease at 42 years. Hypersensitive to loud noises (5 mos.).

Blood pressures: 132/90 (3), *180/130 (4), 118/60 (35), 120/58 (48).

Follow-up blood pressures: *110/80 (5 mos.).

Cold pressor test (5 mos.): 110/80 (control), **160/140 (30 sec.), 140/110 (60 sec.), 120/95 (120 sec.), 120/90 (180 sec.), markedly positive!

Case 22. *H. B.*, J. S. H. #3689 D, 36, white, male. Knocked out 200 yards from ship, unconscious for hours; fractures maxilla, mandible; compound Colles' fracture; ruptured eardrums, draining spinal fluid. Past (Standard Asbestos Co.) and family histories negative for hypertension.

Blood pressures: *120/90 (2), 148/78 (5), 120/80 (6), 105/65 (7), *150/108 (15), 140/70 (19), 130/70 (22), 168/80 (24), 140/90 (25), 150/89 (26), 124/70 (29), 118/68 (48), 148/72 (51), 128/68 (72), 136/82 (96), 120/70 (11 days).

Follow-up blood pressures: *100/70 (4 mos.); 110/80, 122/82 (6 mos.).

Cold pressor test (4 mos.): 100/70 (control), 120/100 (30 sec.), *130/105 (60 sec.), 90/75 (120 sec.), positive.

Case 23. *D. H.*, J. S. H. #3630 D, 36, white, male. Struck down in Service Building of Monsanto Co., lacerations eye (eviscerated). Past history—"low" blood pressure. Family history—father developed sudden heart failure at 55 years. Hypersensitive to loud noises.

Blood pressures: 134/92 (4), *140/100 (5), 140/70 (11), 140/80 (13), 128/60 (15), *140/100 (17), 128/90 (21), 110/80 (30), 136/90 (76, postoperative), 130/80 (79), 120/80 (11 days).

Cold pressor test (11 days): 120/80 (control), *130/110 (30 sec.), 135/105 (60 sec.), 130/100 (120 sec.), positive.

Follow-up blood pressures: 140/88, 128/76 (4 mos.), 120–125/75–80 (7 mos.).

Cold pressor test (7 mos.): 125/80 (control), 145/100, (30 sec.), *155/105 (60 sec.), 120/80 (120 sec.), positive.

Case 24. *E. J. H.*, J. S. H. #3538 D, 37, colored, male. Knocked to ground 100 feet from exploding ship; unconscious for unknown period, lacerations and avulsions of scalp, forearms. Past (U.S. Army) and family histories negative for hypertension.

Blood pressures: *160/90 (6), 108/76 (11), 150/88 (13), 150/92 (15), 150/84 (17), 110/50 (21), 104/66 (23), *136/96 (25), *170/90 (27), 150/85 (29), 140/70 (36), 108/58 (37), 110/85 (38), 118/50 (48), 120/82 (75), 122/82 (85), 118/80, 116/84, 190/70 (7 days, postoperative), 116/78 (8 days).

Cold pressor test (12 days): 120/75 (control), 130/80 (30 sec.), 140/90 (60 sec.), 140/90 (120 sec.), negative or borderline.

Follow-up blood pressures: *140/100 (7 mos.).

Cold pressor test (7 mos.): 140/100 (control), 140/125 (30 sec.), **180/160 (60 sec.), 140/100 (120 sec.), markedly positive!

CASE 25. *C. W. B.*, J. S. H. #3507 D, 21, white, male. Blown off catwalk near Monsanto plant over fence into the water; unconscious for 1–2 minutes, climbed along rocks; fracture of fibula, ruptured eardrums, lacerations. Past history negative for hypertension. Mother and sister hypertensive. Very nervous and hypersensitive to loud noises.

Blood pressures: 120/65 (9), 140/90 (11), *158/96 (14), 124/90 (15), 142/90 (23), *142/110 (25), 130/95 (30), 114/60 (35).

Follow-up blood pressures: 110/80 (4 mos.).

Cold pressor test (4 mos.): 110/80 (control), 125/100 (30 sec.), *135/110 (60 sec.), 130/85 (120 sec.), positive.

CASE 26. *I. F.*, J. S. H. #3676 D, 39, Mexican, male. Blown into tidal waves 200 yards from exploding ship, swam and straddled a floating board; not unconscious, carried an injured boy. Ruptured eardrum draining spinal fluid; linear fracture of skull. Past (T. C. Railroad Terminal Co. and U.S. Army) and family histories negative for hypertension. Admitted from another hospital.

Blood pressures: 144/74 (5 days), 128/80 (6 days), 130/80 (7 days), 130/80 (9 days).

Follow-up blood pressures: *150/110 (4 mos.).

Cold pressor test (4 mos.): 150/110 (control), 170/125 (30 sec.), *180/130 (60 sec.), 160/115 (120 sec.), positive.

CASE 27. *A. P.*, J. S. H. #3582 D, 18, colored, male. Blown off iron pipeline over the bay when ship exploded 50 yards away; managed to reach shore despite extensive laceration of right calf, then became unconscious. Gas gangrene later forced amputation at thigh. Received huge amounts of plasma and blood. NPN rose to 89 mg. % fifth day. Past and family histories negative for hypertension.

Blood pressures: 100/60 (3), 96/50 (6), 120/70 (8), 110/70 (11), 100/52 (15), 84/52 (23), 95/70 (29), 80/50 (34, postoperative), 75/50 (36), 102/60 (37), 110/70 (43), 112/65 (44), *115/94 (45), 102/65 (47), 106/70 (51), 105/75 (52), 110/80 (53), 105/80 (54), 105/75 (55), 110/80 (56), 102/80 (57), 95/70 (58), 102/80 (59), 100/70 (60), 95/70 (61), 120/90 (66, 74), 105/85 (86), 116/80 (96).

Cold pressor test (13 days): *145/98 (control), 150/110 (30 sec.), 145/130 (60 sec.), 160/120 (120 sec.), positive.

Follow-up blood pressures: Not available.

NORMOTENSIVE CASES (6)

CASE 28. *J. J. J.*, J. S. H. #3557 D, 21, colored, male. On dock 50 feet from exploding ship. Unconscious 20 minutes, buried by mud and oil, walked 3–4 blocks, confused 2 days. Pulmonary atelectasis R. L. L.,

ruptured eardrums, lacerations, burn of face. Past history negative for hypertension (W. S. Bellows Co., U.S. Army). Mother 39, hypertensive. Hypersensitive to loud noises.

Blood pressures: 120/64 (6), 116/68 (7), 120/70 (8), 132/82 (9), 128/90 (10), 110/84 (16), 110/82 (17), 108/86 (18), 110/90 (19), *160/90 (20), 132/76 (45), 160/58 (96), *160/94 (12 days).

Follow-up blood pressures: 140/80–85 (5 mos.).

Cold pressor test (5 mos.): 140/80 (control), 150/95 (30 sec.), *140/105 (60 sec.), 140/80 (120 sec.), positive.

Case 29. *R. C.*, J. S. H. #3510 D, 47, white, male. Thrown on floor in 3rd floor cafeteria of Monsanto Co., not unconscious, comminuted fractures both patellae. Past history negative for hypertension (U.S. Army, Monsanto Co.). Father had a stroke. Hypersensitive to loud noises.

Blood pressures: 100/70 (12), 134/80 (17), 112/68 (35), 90/50 and *138/90 (6 days, postoperative).

Cold pressor test (10 days): 114/76 (control), 122/92 (30 sec.), 132/94 (60 sec.), *140/96 (120 sec.), positive.

Follow-up blood pressures: 110/70 (6 mos.).

Cold pressor test (6 mos.): 110/80 (control), 130/110 (30 sec.), *140/125 (60 sec.), 110/80 (120 sec.), positive.

Case 30. *J. R.*, J. S. H. #3637 D, 14, Mexican, male. Collapsing stairs, wooden awning fell on patient fracturing right tibia; lacerations scalp and eye; not unconscious. Past and family histories negative.

Blood pressures: 90/68 (3), 100/60 (4), 110/70 (5), 130/85 (6), *130/90 (7), 120/80 (12), 112/64 (14), 120/80 (24), 110/68 (48), 110/64 (72), 120/90 (9 days).

Follow-up blood pressures: 90/80 (7 mos.).

Cold pressor test (7 mos.): 90/80 (control), 110/85 (30 sec.), 110/90 (60 sec.), 90/75 (120 sec.), negative.

Case 31. *C. Z.*, J. S. H. #4467 D, 4, Mexican, male. At home 8 blocks from explosion, lacerations of face, ruptured eardrums. Past history unknown. Family history negative for hypertension.

Blood pressures: *125/84 (9 days), 112/72 (11 days), 118/76 (12 days), 70/50 (30 days).

Follow-up blood pressures: None obtainable.

Case 32. *J. P. D.*, J. S. H. #3518 D, 37, white, male. Hit half mile from second explosion by piece of steel, crawled half a block before rescued; not unconscious; compound fracture left tibia, lacerations. Past (Monsanto Co., U.S. Army) and family histories negative for hypertension. Hypersensitive to loud noises (4 mos.).

Blood pressures: 120/70 (10), *130/85 (11), 110/64 (12), 108/75 (19), 110/70 (22).

Cold pressor test (10 days): 114/66 (control), 122/94 (30 sec.), 124/106 (60 sec.), *120/110 (120 sec.), positive.

Follow-up blood pressures: 100/70 (4 mos.).

Cold pressor test (4 mos.): 100/70 (control), 130/100 (30 sec.), *140/105 (60 sec.), 95/70 (120 sec.), positive.

Case 33. *R. L. P.*, J. S. H. #3662 D, white, male. Hit by bricks, metal and glass on the 4th floor of Styrene building; not unconscious; ruptured eardrum, draining fluid, compound fracture left humerus, deep lacerations. Post history negative for hypertension (U.S. Navy, etc.). Family history negative.

Blood pressures: 118/68 (6), 110/50 (11), 118/58 (13), 110/50 (14), *142/82 (24, 48), 115/70 (11 days).

Follow-up blood pressures: 100/80 (4 mos.).

Cold pressor test (4 mos.): 100/80 (control), *130/110 (30 sec.), 130/110 (60 sec.), 130/100 (120 sec.), positive.

Summary

Cardiovascular studies conducted on 180 of the blast victims of the Texas City Disaster during the first two weeks of their hospital stay, and on 111 follow-up cases some 4 to 7 months later, revealed certain significant findings.

One hundred and three or 57% of the originally studied 180 cases presented, soon after the blasts, diastolic blood pressure peaks of 95 mm. of Hg. or over. After two weeks of bed rest, only 5 of 61 cases still showed such diastolic elevations. In the follow-up group of 111 cases diastolic blood pressure "minima" of 95 mm. or over were obtained after rest in the sitting position in 24 or 22%. This figure exceeds any others previously noted for comparable age and population groups, even if transient diastolic hypertensive peaks are contrasted to the diastolic minima of the blast victims. Whatever the cause of these phenomena, it is now established that similar transient hypertensive states are followed in a significant number of cases by permanent hypertension with all of its complications. We cannot make definite predictions along this line on our cases without further follow-up studies.

Cold pressor tests were positive in the great majority of blast victims both in the original and in the follow-up series. Numerous cases of marked hyperreaction were noted particularly in the hypertensive subgroups.

Other phenomena resulting from the blasts included hemorrhagic cardiac injuries and electrocardiographic alterations

noted in wartime air blast injuries. Arrhythmias and pulmonary embolism occurred rarely. Transient paradoxical hypertension followed one massive instance of pulmonary embolism in a patient that also hyperreacted to cold.

Conclusions

Hypertension associated with blast injuries occurred as a transient phenomenon in the majority of the victims of the Texas City Disaster and was also present in a significant number of follow-up cases. Cardiac injuries possibly due to air blast were confined to small hemorrhagic areas of the epicardium and myocardium. Electrocardiographic changes possibly due to cardiac blast injuries could not be dissociated from the concomitant states of shock, hemorrhage, and toxemia. Permanent cardiovascular damage due to air blast, if it occurs, is confined to the possible provocation of a hypertensive state in a minority of its victims.

The aid of student assistants C. E. Murtha and H. Rabinowitz is gratefully acknowledged.

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CHEMOTHERAPEUTIC STUDY OF EXPERIMENTAL TOXOPLASMOSIS—PRELIMINARY REPORT*

JOY BARNES CROSS AND LUDWIK ANIGSTEIN

Although it has been twenty-five years since the first case of human toxoplasmosis was reported by Janku (Weinman, 1944), no completely successful treatment has as yet been achieved. Sulfapyridine and sulfathiazole (Sabin and Warren, 1941) still offer the only hopeful therapy but often these drugs only suppress the symptoms. Weinman (1944) reported that in mice, treatment with sulfathiazole or sulfapyridine ". . . cures the disease, but it does not sterilize the infection. The cured mice remain carriers and retain virulent organisms in the brain."

We have considered it advisable in spite of our negative results to make an accounting of our trials for the guidance of future investigators in this field. Sabin's (1942) listing of numerous drugs that have proved ineffective with experimental toxoplasmosis has been useful to us and the present paper makes an addition of streptomycin both alone and in combination with para-amino-benzoic acid, toluidine blue, sulfamylon, para-amino-benzoic acid used in combination with theophylline, and Aralen. It seems reasonable to suppose that the chemotherapy of toxoplasmosis is challenged by the intracellular nature of certain phases of the parasite, and that therefore the choice of a therapeutic agent should be determined by this factor.

The trial of streptomycin is probably the most generally significant. The procedure that Youmans and McCarter (1945) found successful in treating white mice infected with tuberculosis was used. As a paralleling experiment and although Summer's results (1947) were discouraging, streptomycin was used by us simultaneously with PABA mainly because Smadel

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(1947) had found this combination efficacious in certain rickettsial infections.

White mice, weighing approximately 20 grams and from one inbred stock were used. Inoculations were made subcutaneously in the shoulder region and consisted of 0.4 ml. of an emulsion made of brains from two infected mice in 20 ml. of Tyrode's solution. This strain of *Toxoplasma* was secured through the courtesy of Dr. A. B. Sabin and routinely kills in 7 to 8 days following such inoculations. Streptomycin (Streptomycin calcium chloride, Merck) was injected by means of 1 ml. tuberculin syringes, four times daily at six hour intervals in 0.2 ml. doses, each dose containing 750 units. The PABA (Merck) was administered in finely pulverized dog chow and sugar. The latter ingredient constituted 10% of the total food supply and was used because earlier experiments showed that the mice avoided the medicated food unless it was sweetened. An ample supply of food was maintained in specially devised boxes that permitted little wastage, and careful recording of all replenishments were made. At the close of the experiment, the amount of food remaining in the

TABLE 1

Streptomycin

No. of Mice	No. Infected	Therapy	Food Per Day Per Mouse	PABA Per Day Per Mouse	Days Survived
7	7	0	0	0	4 for 7 days 3 for 8 days
7	7	Streptomycin	0	0	5 for 7 days 1 for 7½ days 1 for 8 days
7	7	Streptomycin & PABA	2.4 gm.	1% in food 24 mg.	2 for 6½ days 3 for 7 days 2 for 8 days
7	0	Streptomycin	0	0	Hale and hearty
7	0	Streptomycin & PABA	3.9 gm.	1% in food 39 mg.	Hale and hearty

4 x 750 units per day.
750 units equal 0.2 ml.

boxes was deducted from the total amount recorded. The amount of food per day per mouse and the amount of PABA was calculated from these figures. Anorexia was evident in the infected mice during the last day or so preceding death and accounts for the lessened food intake of that group. It is of interest to note that in both groups of mice that received PABA there was accelerated activity which was still more noticeable in the infected mice. The results of the experiments are shown in Table 1.

Toluidine blue (Fischer No. Nu 1) was found to be ineffective in toxoplasmosis when used in accordance with the procedure of Peterson (1944) which had proved successful with an intake of 5 to 20 mg. per day in curing typhus in mice. Table 2 shows the results of this experiment.

TABLE 2

Toluidine Blue

No. of Mice	No. Infected	Per Cent in Diet	Hours Pre-fed	Drug Per Day Per Mouse	Days Survived
3	3	0	0	0	10
4	4	0.5%	6	15 mg.	2 for 10 2 for 10½
6	6	0	0	0	7
6	6	0.5½	8	18 mg.	5 for 7 1 for 8

Earlier experiments (unpublished) showed that PABA was ineffective. Trials were made using PABA in combination with theophylline etheylendiamine because it has been postulated (Fröhlich and Zak, 1927; Lehman and Dater, 1938) that this compound increased the permeability of the cell membrane. Since the chemotherapeutic resistance of *Toxoplasma* can be attributed perhaps to its intracellular nature, it was hoped that an increase in cellular permeability might expose the parasite to the action of the drug. Theophylline was administered simultaneously with 3% of PABA at the rate of

0.5% in the diet but the period of survival was not appreciably extended. An increase to 1% of theophylline produced symptoms of toxicity.

Although the effective drugs, sulfapyridine and sulfathiazole, are known to be antagonistic to PABA, an investigation of one of the few sulfa drugs not inhibited by PABA (Hamre, 1944) was made. Sulfamylon hydrochloride (4-amino-methylbenzine-sulfonamide hydrochloride) was tried but proved completely ineffective when administered at the rate of 2% in the diet with an intake of 50 mg. per mouse per day. Symptoms of toxicity became apparent when 3% with an intake of 104 mg. per day were used.

The possible resistance of the less accessible intracellular form of *Toxoplasma* suggested the trial of Aralen (7-chloro-4 (diethylamino-1-methylbutylamine) quinoline diphosphate, Winthrop Chemical Company). This compound also appears under the code number of SN-7618 and has been called chloroquine. (Loeb, 1946; Wiselogle, 1946). Aralen is one of the newer antimalarials which is believed to attack the "tissue phase" of the malarial parasite. When Aralen was administered as 0.1% of the food, the survival period of the infected mice was extended slightly, but this dosage was evidently toxic. One-half of the that dosage was non-toxic but ineffective. Intermittent dosage with 0.1% and 0.05%, and continuous dosage with 0.075% were all unsuccessful. The results of the use of 0.075% are shown in Table 3.

TABLE 3

Aralen (Chloroquine)

No. of Mice	No. Infected	Per Cent in Diet	Days Survived Drug	Days Survived Infection
3	3	0	0	1 for 7 2 for 7½
4	4	0.075	1 for 11 2 for 12 1 for 13	1 for 8 2 for 9 1 for 10
3	0	0.075	2 for 9 1 for 16	0

SUMMARY

The following chemotherapeutic agents were tested in experimental toxoplasmosis in mice; Streptomycin (3,000 units per day), used both alone and simultaneously with PABA; PABA in combination with theophylline; Toluidine blue; Sulfamylon; Aralen (SN 7618). The results showed no significant differences in the survival rates of the treated mice as compared with the untreated controls.

The authors are grateful to Professor E. J. Poth, Department of Surgery for supplying us with sulfamylon, to Dr. P. L. Ewing, Department of Pharmacology for advice on the administration of theophylline; to Mr. E. P. Read and Mr. H. Joseph for technical assistance with the experiments; to Merck Chemical Company for furnishing us with streptomycin, and to Winthrop Chemical Company for supplying us with Aralen.

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Calling Attention To

Items of possible interest to friends of our effort

March, 1948

1. Books! Books! Books!: OSRD boys point with pride to Advances in Military Medicine, edited by E. D. Andrus and others (Little, Brown, Boston, 1948, 2 vols., 900 pp., $12.50). J Read interests with *Humour and Humanism in Chemistry* (Bell, London, 1948, 400 pp., 21s). R. A. Willis offers *Pathology of Tumours* (Butterworth, London, 1948, 1068 pp., 63s). R. Abder halden discusses *Medizinische Terminologie* (Schwabe, Basel, 1948, 640 pp., 32 SwFr). J. M. Hill & W. Dameshek edit *The Rh Factor* (Sp. Issue No. 2, *Blood*, Grune & Stratton, N.Y., 1948, 198 pp., $4.25). W. F. Petersen regards *Man, Weather, Sun* (C. C. Thomas, Springfield, Ill., 1948, 800 pp., $10.50). Have you seen the attractive American Lecture Series?: G. H. Whipple, *Hemoglobin, Plasma Protein and Cell Protein* (1948, $1.40); L. T. Samuels, *Nutrition and Hormones* (1947, $1.65); C. R. Moore, *Embryonic Sex Hormones and Sexual Differentiation* (1947, $2); H. T. Karsner, *Acute Inflammation of Arteries* (1947, $0.90); E. I. DuBois, *Fever Regulation of Body Temperature* (1948, $2); W. D. Forbus, *Granulomatus Inflammation* (1948, $0.90); H. W. Magoun, *Spasticity, Stretch Reflex and Extra Pyramidal Systems* (1948, $2); I. H. Page and A. C. Corcoran, *Experimental Hypertension* (1948, $2); D. T. Smith, *Fungus Diseases of the Lungs* (1948, $2), and H. G. Wolff and S. Wolf, *Pain* (C. C. Thomas, Springfield, Ill., 1948, 94 pp., $2). F. A. Beach reviews *Hormones and Behavior* (Hoeber, N.Y., 1948, 382 pp., $6.50). R. T. Williams covers *Detoxication Mechanisms* (Wiley, N.Y., 1948, 288 pp., $5.50). J. Alexander huffs on *Life: Its Nature and Origin* (Reinhold, N.Y., 1948, 298 pp., $5).

2. Pharmacological, Etc.: Over a thousand abstracts appear for March *Federation Proceedings*, from B. E. Abreau and others on cerebral blood flow to M. Zweig and others on tetraethyl ammonium on gastric function (*Fed. Proc.*, 7:1–387, 1948), with much Blakian representation, but little on theoretical analysis or synthesis. C. C. Pfeiffer offers interesting hypothesis on nature of spatial relationships of chemical groups required for maximal muscarinic action (*Science*, 107:94, Jan. 23, 1948). N. P. Sullivan and others note slow absorption and prolonged high blood levels from injection of procaine-penicillin G (*Science*, 107:169, Feb. 13, 1948). A. Albert and R. Goldacre suggest hygrogen bonding as factor in antibacterial action of acridines (*Nature*, 161:95, Jan. 17, 1948). R. Pratt and others show that traces of cobalt enhance penicillin action (*J. Bact.*, 55:75, 1948). A. Voureka shows that penicillin resistant staphlococci become sensitive again after contact with other organisms (*Lancet*, 1:62, Jan. 10, 1948). M. Block and H. M. Pollard recommend sulfathalidine for chronic ulcerative colitis (*Gastroenterol*, 10:46, 1948). J. Vaughan recommends

high protein intake in anemia from trauma and sepsis (*Brit. Med. J.*, 1:36, Jan. 10, 1948). Z. Z. Godlowski notes value of insulin hypoglycemia in allergic bronchial asthma is due to adrenin secretion (*ibid.* p. 46). W. L. Lipschitz and E. Stokey find formoguanamine (5 mgs./kg.) a potent safe diuretic by mouth (*J. Pharmacol.*, 92:131, 1948). G. Brownlee and S. R. M. Bushby report on chemotherapy and pharmacology of aerosperin, a more potent and selective gram negative antibiotic than streptomycin (*Lancet*, 1:127, Jan 24, 1948). Our C. R. Allen and others describe variations in signs of acute oxygen want in anesthesia (*Anesth.*, 8:601, 1947).

3. ETC.: Some faces should redden over C. A. Mills' report on distribution of American research funds (*Science*, 107:127, Feb. 6, 1948), pointing up another factor in establishment of a provincial scientific canon, with which it is heresy (and starvation) to disagree, since the distributors of American scientific funds also control our national scientific societies and scientific journals. C. L. Walker skillfully confirms Malthus's gloomy predictions and suggests steady drive for birth control addressed to world's women as only possible escape (*Harper's*, 196:97, Feb., 1948). A. S. McNalty notes influence of medicos on English poetry (*Med. Bookman*, 2:15, 1948). P. S. Hench and others review rheumatism and arthritis reports (*Ann. Int. Med.*, 28:66, 1948). E. W. Dunklin and T. T. Puck show low viability of air-borne bacteria at 50 per cent humidity (*J. Exp. Med.*, 87:87, 1948). N.B., fine discussion by F. M. Burnet of basis of allergic disease (*Med. J. Austral.*, 1:29, Jan. 10, 1948) and on the natural history of tuberculosis (*ibid.*, p. 57, Jan. 17, 1948). J. E. P. Toman and others discuss electroconvulsion phenomena, finding multiple seizures on continuous stimulation (*J. Neuropath. Exp. Neurol.*, 7:35, 1948). B. Taylor and others find X-rays shorten long fibrous particles of thymus nucleate (*Arch. Biochem.*, 16:19, 1948). M. F. Perutz shows freedom of rotation of hemoglobin molecules in red blood cells ensures high rates of oxygen reaction and diffusion (*Nature*, 161:204, Feb. 7, 1948). Our A. Ruskin, O. W. Beard and R. L. Schaffer discuss blast hypertension in relation to Texas City disaster victims (*Am. J. Med.*, 4:228, 1948).

APRIL, 1948

1. POLICY: New format in deference to presbyopic tendencies of older readers. No references ordinarily to articles in *Journal American Medical Association*, since assumption is that all readers of this read that regularly. Ditto for *Texas Reports on Biology and Medicine*. Highly technical articles are noted only when results appear to have far-reaching significance.

Punctuation abbreviated to conserve space and promote easy reading.

2. CULTURAL?: G. Jefferson raises some interesting questions in his Scepsis Scientifica (*Brit. Med. J.*, 1:379, Feb. 28, 1948). F. T. Gardner is broadly amusing in her " Bells Toll for Primes: An Inquiry Into the Fate of William the Conqueror" (*Surg. Gyn. and Obs.*, 86:377, 1948). L. H. Butterfield describes Benj. Rush (1745–1813) as promoter of useful

knowledge (*Proc. Amer. Philosoph. Soc.*, 92:26, 1948). Fittingly appropriate in Ward Ritchie composition is E. Belt, K. T. Steinitz, and M. Archer's *Manuscripts of Leonardo da Vinci: Their History with a Description of the Manuscript Editions in Facsimile* (Elmer Belt Library of Vinciana, Los Angeles, 1948, 70 pp.). D. L. Miller analyses the effect of evolutionary concepts on scientific method (*Philosoph. Sci.*, 15:52, 1948). L. C. Strong and E. L. McCawley verify 16th Century use of oil of spindle (Euonymus europeaeus), honey and pitch, decoded from Voynich Mss., as contraceptive (*Bull. Hist. Med.*, 21:898, 1947). M. Denny gives letters to Linnaeus from Alexander Garden from Charleston, S.C., between 1755 and 1773; gardenia named for him and he probably inspired W. C. Wells (1757–1817), pioneer meteorologist and evolutionist (*Isis*, 38:161, 1948). M. Grene exposes absurdities and recognizes valid contributions of existentialists Sartre, Heidegger, Kierkegaard, Marcel, and Jaspers (*Dreadful Freedom: A Critique of Existentialism*, Univ. Chicago Press, 1948, 150 pp., $2.75). Important are H. Simons' posthumous essays *Economic Policy for a Free Society* (Univ. Chicago Press, 1948, 353 pp., $3.75). P. Schrecker philosophizes on *Work and History* (Princeton Press, 1948, 350 pp., $5).

3. PHARMACOLOGICAL?: T. H. Jukes and E. L. R. Stokstad survey pteroylglutamic acid and related compounds (*Physiol. Rev.*, 28:51, 1948). H. Speert discusses local action of sex hormones (*ibid.*, p. 23). F. J. Stare and others continue studies on fat emulsions for intravenous nutrition (*J. Lab. Clin. Med.*, 33:143–180, 1948). L. P. Garrod emphasizes bactericidal action of streptomycin (*Brit. Med. J.*, 1:382, Feb. 28, 1948). B. A. Rubin reports on trypanocidal effects of antibiotic lactones (*Yale J. Biol. Med.*, 20:233, 1948). G. I. M. Swyer observes anti-histamine effect of Na salicylate and its antagonism to snake venom capillary damage (*Biochem. J.*, 42:28, 1948). M. R. Castex, L. E. Camponovo, and F. E. Labout recommend 5 cc. 1% succinic acid IV as useful respiratory stimulant (*La Prensa Med. Argent.*, 35:195, Jan. 30, 1948). P. Fantl and M. H. Nance describe a heat labile globulin prothrombin accelerator (*Med. J. Austral.*, 1:128, Jan. 31, 1948). G. Calcutt and J. P. Newhouse find cysteine and serine inhibit photodynamic action of benzpyrine (*Nature*, 161:53, Jan. 10, 1948). S. C. Bukantz and G. J. Dammin suggest fluorescein in skin tests as indicator of antihistamine action (*Science*, 107:224, Feb. 27, 1948). R. G. Horton and others show high potential skin toxicity of benzene hexachloride ("Gammexane") as insecticide in clothing (*Science*, 107:246, March 5, 1948). G. L. Hobby and others discuss procaine penicillin (*Proc. Soc. Exp. Biol. Med.*, 67:6, 1948). D. Scherf finds fagarine (fr. Argentine Fagara coco) effective in controlling auricular fibrillation (*ibid.*, p. 59).

4. NOTABLE?: D. M. Horstman discusses epidemiology of polio (*Lancet*, 1:273, Feb. 21, 1948). R. Mosera notes marked rise of systolic blood pressure in asthmatic attacks (*Arch. Uruguay Med. Cir. Esp.*, 30:605, 1947). Note important symposium on lactation and milk by K. C. Richardson, J. H. Burn, and others (*Brit. Med. Bull.*, 5:120–219, 1948).

W. Baumgartner describes phagocytosis of red cells (*Helv. Med. Acta.*, 15:61, 1948). Excellent is C. W. Walter's *Aseptic Treatment of Wounds* Macmillan, N.Y., 1948, 372 pp., $10). S. Cobb well reviews century's development in neurology, psychiatry, and neurosurgery (*Arch. Neurol. Psychi.*, 59:63, 1948). W. H. Mathews recommends examining sputum for tumor cells in suspected lung cancer (*Canad. Med. Asso. J.*, 58:236, 1948). F. Hawking describes tissue culture of Leishmania donovani (*Trans. Roy. Soc. Trop. Med. Hyg.*, 41:545, 1948). Q. H. Gibson analyses enzyme systems in red cells responsible for reduction of methb (*Biochem. J.*, 42:13, 1948). D. C. Darrow discusses electrolyte metabolism in man (*Bull. N.Y. Acad. Med.*, 24:147, 1948). N. W. Shock describes metabolism in old age (*ibid.* p. 166). R. Doerr offers 2 vols., *Die Immunitatsforschung*, Bd. 1, *Antikorper*, Bd. 2, *Komplement* (Springer, Wien, 268 and 79 pp., 1947, SwFr 32). H. T. Clarke, J. R. Johnson, and R. Robinson edit NAS *Chemistry of Penicillin* (Princeton Press, 1948, 1200 pp., $30). Sympathy to AMA on printing troubles!

MAY, 1948

1. CLINICAL: J. C. Waterlow discusses *Fatty-Acid Disease in Children* (Med. Res. Council Rep., 263, London, 1948, 2s/2d). M. Zehnder describes diagnostic use of vasokymogram in studying passage of fluid on same X-ray film (*Helv. Clin. Acta.*, 15:65, 1948). I. J. Wood and others discuss biochemistry and histology of hepatitis (*Med. J. Austral.*, 1:249, Feb. 28, 1948). A. C. Corcoran, R. D. Taylor, and I. H. Page review functional patterns in renal disease (*Ann. Int. Med.*, 28:560, 1948). H. Rasmussen and G. Nyhus describe EKG in mitral stenosis (*Acta. Med. Scand.*, 129:446, 1948). V. H. Moon surveys the pathology of secondary shock (*Am. J. Path.*, 34:235, 1948). Our A. H. Gamble, H. F. Wiese, and A. E. Hansen note possible cardiac effect of hypokalemia in prolonged diarrhea (*Pediatrics*, 1:58, 1948). Our A. Ruskin, O. W. Beard, and R. L. Schaffer report on blast hypertension in Texas City disaster patients (*Am. J. Med.*, 4:228, 1948). P. Heinbecker offers his idea of pathogenesis of diastolic hypertension (*Surgery*, 23:618, 1948).

2. MICROBIOLIGICAL AND ANTIBIOTICAL: M. S. Marshall offers *Applied Medical Bacteriology* (Lea & Febiger, Phila., 1948, 340 pp., $4.50). S. D. Elek describes methods for in vitro recognition of toxigenic bacterial strains (*Brit. Med. J.*, 1:493, March 13, 1948). E. F. Gale discusses synthesis of proteins by micro-organisms (*Chem. Indust.*, 9:131, Feb. 28, 1948). W. van Iterson reports electron microspial findings on bacterial cytology (*Biochim. Biophys. Acta.*, 1, 527, 1948). I. M. Dawson and A. S. McFarlane describe structure of vaccinia virus (*Nature*, 161:464, March 27, 1948). D. J. Bauer suggests xanthine oxidase and pyruvic dehydrogenase are essential for virus growth (*Brit. J. Exp. Path.*, 28:440, 1948). G. M. Richardson suggests relation between World War I use of mustard gas and influenzal

epidemic of 1918 (*New Zealand Med. J.*, 47:4, 1948). R. K. Callow and others report antibiotic fr. bavillus lichenformis active vs. M. tuberculosis, but may injure kidney (*Brit. J. Exp. Path.*, 28:418, 1948). R. M. Smith and others report biological studies on chloromycetin (*J. Bact.*, 55:425, 1948). G. E. Boxer and others describe chemical estimation of streptomycin in blood, finding it proportional to dosage with rate of decrease proportional to concentration (*J. Pharmacol.*, 92:226, 1948). R. Pratt and J. Dufrency give cytochemical interpretation of mechanism of penicillin action (*Bact. Rev.*, 12:79, 1948). G. Rake discusses streptomycin as an essential nutrilite (*Proc. Soc. Exp. Biol. Med.*, 67:249, 1948).

3. PHARMACOLOGICAL: C. S. Jang and others note antimalarial action of Dichroa febrifuga (Chang Shan) root, due to quinazoline derivative (*Nature*, 161:400, March 13, 1948). B. Ekman says Ascorbic acid and cucumber are helpful in chronic benzene poisoning (*Acta. Pharmacol. Tox.*, 3:261, 1947). G. Brownlee and others describe sulphetrone, a diaminodiphenyl-sulphone, as useful in tuberculosis (*Brit. J. Pharmacol.*, 3:15, 1948). K. Bailey and S. V. Perry discuss role of sulphydryl in interaction of myosin and actin (*Biochim. Biophys. Acta.*, 1:506, 1947). In appraising carminative, S. Alstead and J. F. Patterson conclude pituitrin IM is best for flatulence, provided rectum is empty (*Lancet*, 1:437, March 27, 1948). R. Carratala studies clinical and experimental toxicity of aspirin (*La Prensa Med. Argentina*, 35:231, Feb. 6, 1948). L. Biro shows B Complex favorably influences adrenal insufficiency in infections (*Schw. Ztschr. Path. Bact.*, 11:59, 1948). C. Heymans and G. R. Vloeschhonwer describe phenylcyclopentane cmpd., "parpanit" as active broncho and enteric spasmolytic agent (*Arch. Internat. Pharmaco. Therap.*, 75:307, 1948). R. Parmentier and P. Dustin discuss mitotic poisoning and radimimetic action of hydroquinone (*Nature*, 161:527, April 3, 1948). S. S. Kety and others find nitrous oxide solubility same in blood and brain (*J. Biol. Chem.*, 173:487, 1948). E. Boyland reviews chemical carcinogenesis and cancer chemotherapy (*Yale J. Biol. Med.*, 20:321, 1948). L. W. Roth and others describe pyridyl-thenyl cmpd., "thenylene" as potent non-toxic anti-histamine agent clinically useful in allergy (*Arch. Internat. Pharmaco. Therap.*, 75:362, 1948). G. Lehman reports on antihistamine action of "thephorin" new pyridindene derivative (*J. Pharmacol.*, 92:249, 1948). Note symposium on insecticides in relation to food production (*Indust. Eng. Chem.*, 40:673–717, 1948). K. P. DuBois and others report rodenticidal action of "castrix," a chloro pyrimidine (*Proc. Soc. Exp. Biol. Med.*, 67:169, 1948). D. I. Macht reports phytopharmacological experiments on urethane (*ibid.*, 237).

4. NEUROLOGICAL: G. Weddell and others note association of unpleasant pain reactions from skin with reduction in usual peripheral pattern of nerve distribution (*J. Neurophysiol.*, 11:99, 1948). M. A. Rothenberg and others show by tagged N that acetylcholine does not pass across nerve membrane, allowing no escape during conduction, limiting pharmacological

action to synapse (*ibid.*, 110). J. Piatt discusses form and causality in neurogenesis (*Biol. Rev. Cambridge Philosoph. Soc.*, 23:1, 1948). W. E. L. Clark and W. E. Russell discuss form and function of frontal lobes (*Lancet*, 1:353–360, March 6, 1948). H. B. Shumacker and others review causalgia and vasomotor disturbances (*Surg. Gyn. Obs.*, 86:452, 1948). J. T. Roberts reports on role of vasa nervorum (*Am. Heart J.*, 35:369, 1948). A. J. Arieff and others analyse complicated emotions in neighborhood quarrels (*J. Nerv. Ment. Dis.*, 107:62, 1948).